Vol. 50, No. 8, March, 1961
Russian Original Dated August, 1960

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU

INTRAARTERIAL INFUSION OF PROCAINE

IN THERAPEUTIC PRACTICE

by N. K. Gorbadei

Translated from Russian

This English translation of Dr. Gorbadei's book includes as an appendix a report by I. I. Velikanov on treatment of hypertensive patients with intraarterial procaine infusions. Dr. Velikanov's report was first published in the Bulletin of the Academy of Sciences of the Kazak SSR.

Cases of peptic ulcer dating back to the siege of Leningrad responded to a series of infusions of procaine into the arteries, so that the patients were able to resume their normal activities, according to the author, Dr. N. K. Gorbadei who has reported on 177 cases of peptic ulcer treated at the Leningrad Sanitary-Hygiene Medical Institute.

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Intravascular Infusion of Procaine in Therapeutic Practice (a survey of the literature) (A Short Account of the Properties of Procaine; Intravenous Procaine Infusions; Intraarterial Procaine Infusions.)

The Mechanism of Action of Procaine

The Technique of Intraarterial Infusion of Procaine

The Treatment of Peptic Ulcerations by Intraarterial Procaine Infusions [Clinical Features of the Patients Under Investigation; Influence on Subjective Symptoms; Roentgenological Findings During Treatment; Acid-secreting Function of the Stomach During Treatment; Changes in the Blood Picture; Changes in the Zones of Cutaneous Sensation; State of the Cardiovascular System (Electrocardiography and Plethysmography) During Treatment.]

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Supplement

The Treatment of Patients with Hypertension by Intraarterial Infusion of Procaine Solution. I. I. Velikanov

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EXPERIMENTAL BIOLOGY AND MEDICINE

A translation of BYULLETEN' EKSPERIMENTAL'NOI BIOLOGII I MEDITSINY, a publication of the Academy of Medical Sciences of the USSR

(Russian Original Dated August, 1960)

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The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

FLUCTUATIONS IN HEART RATE CAUSED BY PROLONGED SYMPATHETIC STIMULATION

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(Presented by Active Member AMN SSSR S. E. Severin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 3-8, August, 1960
Original article submitted June 2, 1959

A great deal of attention has been paid to the problem of the two opposite effects mediated by the nerves supplying the heart. A number of facts have been accumulated concerning the action of the vagus on the work of the heart. Some authors attribute the biphasic influence of this nerve to the liberation of acetylcholine [4, 5, 8, etc.], while others explain it in terms of the opposed effects of acetylcholine and adrenalin [1, 7, 9, and others]. There are some results which indicate that the prolonged action of acetylcholine brings about the liberation of its antagonist adrenalin, through the occurrence of internal compensatory changes; the reverse relationships were obtained for the action of adrenalin [10 and others].

In this context, the Sechenov-Mechnikov phenomenon is of special importance; the effect is that prolonged stimulation of the vagus for up to 40 minutes induces in the frog heart periods of depression alternating with recovery. Experiments in our laboratory [2, 3] have established that, during the depressive phases, acetylcholine is excreted, and during recovery, adrenalin is formed.

In the present article we report results of experiments performed to explain the nature of the effect of prolonged sympathetic nerve stimulation on the heart, and to determine the neurohumoral changes which take place in cardiac muscle in these circumstances.

METHOD

Experiments were carried out on Rana temporaria hearts isolated by Straub's method. Stimulation was applied at the level of the second and third sympathetic ganglia; sawtoothed pulses were applied at a frequency of 20 cps, and at a voltage which was adjusted separately for each experiment. Parallel experiments were set up to determine the influence of certain drugs applied during prolonged sympathetic stimulation. (Details of the concentration of the substances used will be given when the experiments are described.)

RESULTS

It was found that, during prolonged sympathetic stimulation, the Sechenov-Mechnikov phenomenon occurs, that is to say, there are phasic changes in the activity of cardiac muscle, as shown by phases of increased and decreased output. Figure 1 shows the results of one typical experiment. In all phases of increased activity following prolonged stimulation, the heart rate was higher than normal. Cessation of the stimulation always caused a return to normal heart work.

Having obtained a biphasic sympathetic effect, we decided to determine, by applying certain drugs, the neurohumoral changes accompanying prolonged sympathetic stimulation, and to find what part was played by these changes in the occurrence of the different phases of activity of cardiac muscle. For this purpose we studied the action of ergotamine, atropine, and ascorbic acid on the periodicity.

Action of ergotamine. From our own and from published results, it might be expected that the origin of a period of increased heart activity is due to the periodic appearance in the heart of adrenalinlike substances. To test this hypothesis, an injection of $1 \cdot 10^{-4}$ ergotamine was made into a heart which was exhibiting the periodicity effect. Such an injection will eliminate the normal action of adrenalin. Figure 1 shows the results of a typical experiment. The kymogram shows clearly the influence of the drug on the phasic changes of cardiac activity produced by prolonged sympathetic stimulation. Five to six minutes after injecting the substance, the heart usually stopped for a long period, and began again only when stimulation ceased.

The elimination by ergotamine of the active phase indicates evidently that adrenalinlike substances are concerned in inducing this phase. According to some Hungarian workers [9 and others], ergotamine does not eliminate the secondary effect of adrenalin, which is due to the liberation of acetylcholine. From this result it might be concluded that the prolonged arrest of the heart by ergotamine, given together with sympathetic stimulation, might be due to the accumulation of acetylcholine. However, according to Kh. S. Koshtoyants, it is possible that oxidation products of adrenalin and, in particular, adrenoxin, may be concerned in the development of the

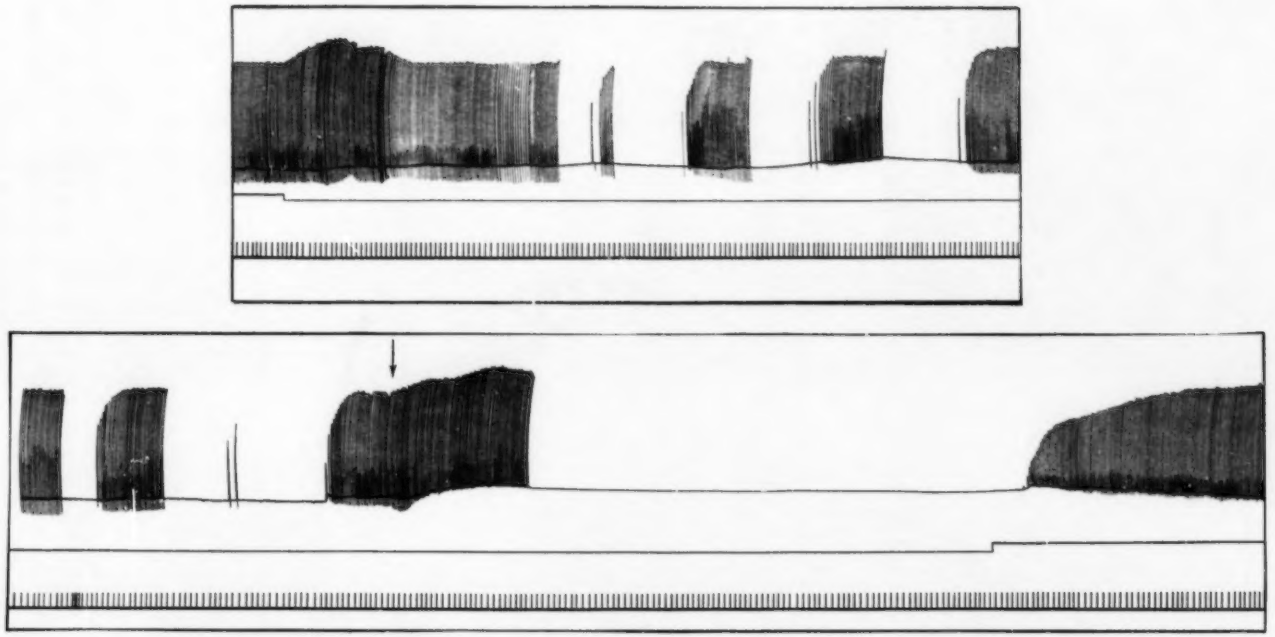

Fig. 1. Effect of ergotamine on the periodic activity of the heart during prolonged sympathetic stimulation. Phases of increased and decreased cardiac activity can be seen. Curves, from above downward. Contraction of cardiac muscle; stimulus marker; 5-sec time-marker; the arrow indicates moment of injection of ergotamine (concentration $1 \cdot 10^{-4}$). Strength of stimulus 11 v.

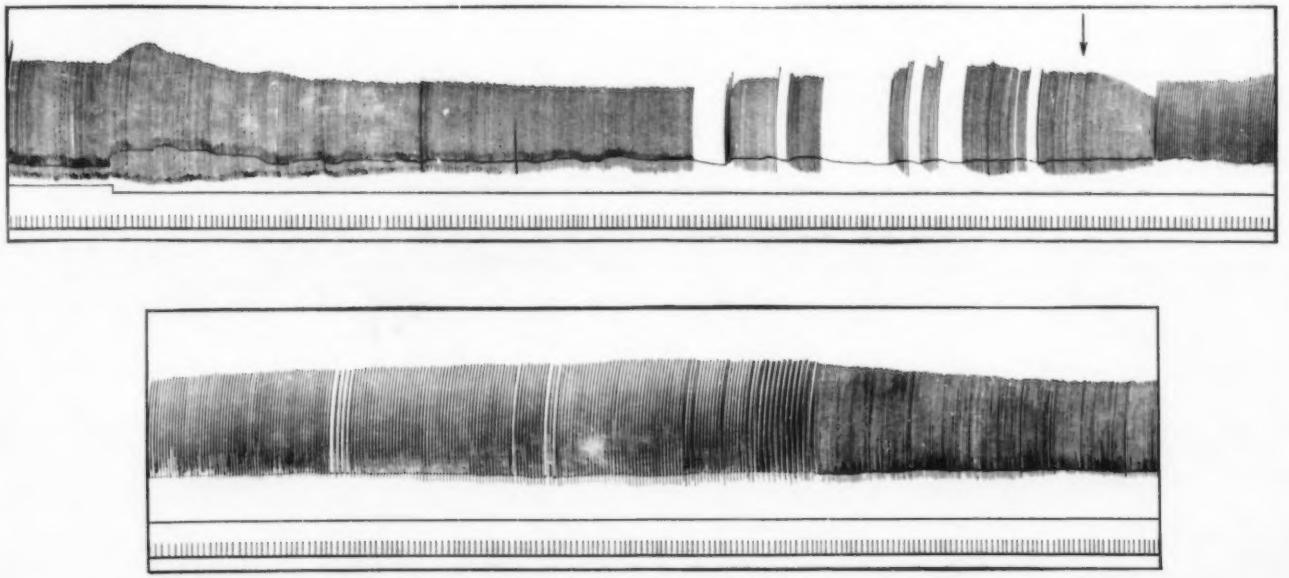

Fig. 2. Effect of atropine on the periodic activity of the heart during prolonged sympathetic stimulation. Curves, from above downward: Contraction of cardiac muscle; stimulus marker; 5-sec time-marker; the arrow indicates the injection of atropine (concentration $1 \cdot 10^{-4}$). Stimulus strength 10 v.

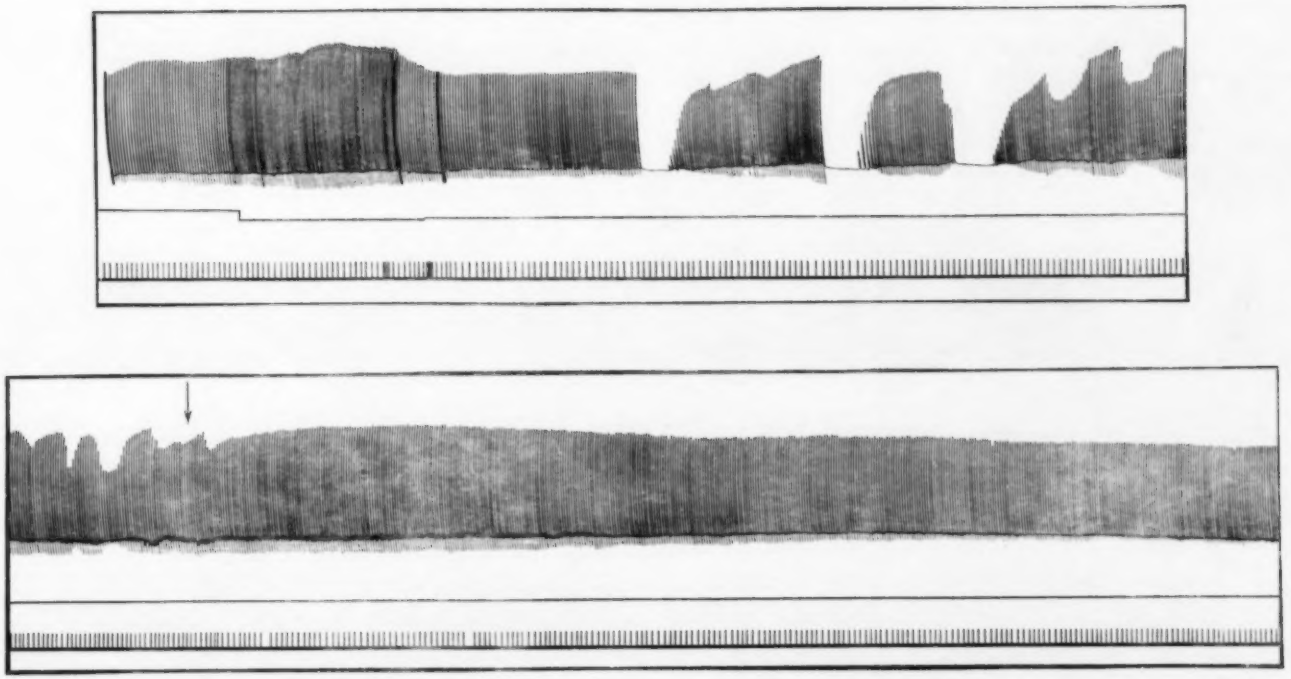

Fig. 3. Effect of ascorbic acid on the periodic activity of the heart during prolonged sympathetic stimulation. Curves, from above downward: Contraction of cardiac muscle; stimulus marker; 5-sec time-marker; the arrow indicates the injection of ascorbic acid (concentration $1 \cdot 10^{-3}$). Stimulus strength 8v.

depressed phase. Here we may note that ergotamine enhances the depressive action of adrenoxin [6].

Both hypotheses have been examined experimentally. To test the possibility that acetylcholine was concerned in the production of the depressive phases, we carried out a number of experiments with atropine.

Action of atropine. We used $1 \cdot 10^{-5}$ atropine in order to alter the action of acetylcholine. In Fig. 2 are shown the results of a typical experiment. From the kymogram it can be seen that atropine, injected after periodic activity has been established by prolonged sympathetic stimulation, eliminates the depressed phase, but that nevertheless the return toward normal is not complete. The curve shows a marked reduction in rate to half the normal value. These results show that there must be some additional influence responsible for the depressed phase.

To test the hypothesis that oxidation products of adrenalin are responsible for the depressed phase, we carried out a number of experiments designed to study the action of ascorbic acid on the heart during prolonged sympathetic stimulation.

Action of ascorbic acid. Our reasoning was based on the fact that ascorbic acid will stabilize any sympathetic effect on the heart by preventing the oxidation of adrenalin. Having established periodic phases of decreased and increased heart output, we introduced $1 \cdot 10^{-3}$ ascorbic acid through a cannula. In Fig. 3, it can be seen that, after the substances have been introduced, the periodicity ceases, because the depressed phase has been eliminated; there is also a characteristic increase in amplitude. Under these circumstances, continuation of the stimulus brings about no changes either in the amplitude or the rate of the contractions.

From this set of experiments, we obtained a clear idea as to the part played by adrenalin oxidation products in initiating the depressed phase, and found that ascorbic acid, which stabilizes the oxidation of adrenalin, completely eliminates it. This result is in agreement with the statement of Kh. S. Koshtoyants that during prolonged sympathetic nerve stimulation, it is not the adrenalin-like substances themselves, but their oxidation products, which bring about the liberation of acetylcholine.

Our experiments have demonstrated that during prolonged sympathetic stimulation, there is an alternation of phases of stimulation and suppression of the heartbeat (Sechenov phenomenon). The phenomenon is apparently due to periodic biochemical changes which occur as a result of the prolonged action of adrenalinlike substances and their oxidation products.

SUMMARY

Experiments on isolated Rana temporaria hearts were carried out in order to determine the effect of prolonged sympathetic nerve stimulation on cardiac action, and to study the neurohumoral shifts in cardiac muscle produced. The sympathetic chain was stimulated for 30 minutes at the second and third ganglia. In several experiments, it was found that periodic changes in cardiac muscle activity occurred, as shown by periods of increased and decreased cardiac action. The intensified phase was associated with the appearance of adrenalinlike substances in the heart, while the depressed phase was connected first, with the appearance of acetylcholine, and secondly, with that of adrenalin oxidation products. Thus, the phasic changes induced by prolonged sympathetic nerve stimulation are evidently due to periodic biochemical changes caused by the prolonged action of adrenalinlike substances and their oxidation products.

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*See English translation.

THE RELATION BETWEEN IMPULSE AND TONIC COMPONENTS IN THE ELECTROENCEPHALOGRAM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 9-12, August, 1960
Original article submitted November 3, 1959

The electroencephalographic method is widely used in both neurophysiological and clinical investigations. But in electroencephalographic theory there is so far no unanimous opinion as to the nature of the bioelectric processes that are recorded with the electroencephalograph (i.e., those variations in electrical potential of the brain whose frequency spectrum lies between 0.3 and 100 cps).

The idea, first put forth by E. Adrian and B. Matthews in 1934 [6], and supported by subsequent investigations of E. Adrian and G. Moruzzi in 1939 [7], that the electroencephalogram is the summed effect of the activity of a large number of nerve cells, continues to hold sway at the present time. On the basis of this hypothesis, electroencephalographic phenomena began to be interpreted as manifestations of synchronous or asynchronous activity of neurons. It was on the basis of this idea that such concepts as "synchronization," "hypersynchronization," and "desynchronization" came into the practice of electroencephalography. Whereas synchronization and hypersynchronization reflect (in the opinion of the proponents of this view) different degrees of synchronousness in the activity of a large number of nerve cells, desynchronization is supposed to reflect asynchronous electrical activity of nerve elements.

The spontaneous rhythmic activity that can be recorded from a nerve cell with a microelectrode would seem to be confirmation of this hypothesis. But it is necessary to explain how the extremely delicate coordination of the activity of a large number of neurons is accomplished in order to create, as a result of algebraic summation of single cell discharges (the duration of a cell discharge is equal to 1 msec), the quite prolonged electroencephalographic wave (for example, the alpha wave, which lasts about 100 msec).

In order to explain the mechanisms of synchronization of neuron activity, a number of theories have been proposed which make it possible to understand this process to one degree or another: the chemical theory of H. Hoagland [14], the neuron theory of J. Eccles [11], the electrical theory of R. Gerard and B. Libet [13], and A. Fessard's theory [12] of the presence of a special pacemaker.

Alongside this viewpoint, there exists another view of the nature of electroencephalographic variations, whose adherents believe that these variations are an independent process, and not the summed effect of the impulse activity of nerve cells. Thus, V. S. Rusinov suggests that the principal forms of bioelectric phenomena in the cortex—delta, theta, alpha, beta, and gamma waves — represent an independent process and reflect local excitation of neurons or groups of neurons [3].

In support of the idea that electroencephalographic variations are not the summed effect of the impulse activity of nerve cells, a number of investigations carried out with the utilization of microelectrode techniques could be cited. The authors who made these investigations were unable to find a clear-cut relation between changes in the electroencephalogram and changes in the impulse activity of single neurons at the same site in the brain [8, 9, 10, 15].

But an analysis of the activity of a single neuron and of the over-all electroencephalogram recorded from the same point on the cortex still does not give us the right to reject the view that the electroencephalogram is the summed effect of the impulse activity of a large number of neurons, since it is always possible to assume that the very neuron being studied is for some reason not synchronized in activity with the other neurons, and consequently, has no substantial effect on the production of the electroencephalographic wave.

Our task, therefore, was to characterize the impulse activity of as many neurons as possible at the site from which the gross electroencephalogram was being recorded, and to show what sort of correlation there is between the gross electroencephalogram and the im-

pulse activity of neural elements situated at the point of recording of the electroencephalogram.

METHODS AND RESULTS

The microelectrode technique, which permits us to study successfully the electrical activity of a single neuron, becomes inconvenient when it is necessary to determine the electrical activity of many nerve elements (tens or hundreds) in a small volume of tissue. It is difficult to introduce more than 2-3 microelectrodes into a small region of the brain, but more important, a large number of amplifiers, as well as of recording channels, are required; this complicates the investigation and sometimes makes it impossible. In looking for an alternative procedure, we began with the following considerations:

1. The nerve cell discharge has the form of an impulse lasting 0.8-2 msec [3, 16]. Consequently, to record cell discharges, it is necessary and sufficient to use an amplifier with a transmission band of roughly 500-3000 cps.

2. A nerve cell discharge can be recorded not only by introducing an electrode into the cell, but also in the case where the recording electrode is some distance away from it. But in this case, the amplitude of the electrical discharge drops markedly as the distance from the cell increases [17]. Thus, if we selectively amplify the impulse component, it will be possible to record it, using conventional electrocorticographic electrodes.

As a result, we used an electrocorticographic electrode with a recording surface of 1-2 mm^2, and a biological-current amplifier with a transmission band of 300 to 10,000 cps; but, since we employed an ordinary standard ac amplifier with a transmission band of from 2 to 10,000 cps for this purpose, the lower end of the band was raised with a capacitative filter. For recording the electroencephalogram, on the other hand, the signal was taken from a different output of the wide-band amplifier (i.e., without restricting the range). Thus, two signals were taken from the wide-band amplifier: one containing frequencies between 2 and 10,000 cps (electroencephalographic) and the other containing frequencies between 300 and 10,000 cps (impulse). These two signals were fed into different inputs of a double-beam cathode-ray oscillograph and recorded on photographic film. Utilization of the cathode-ray oscillograph was necessitated by the high frequency of the impulse process being studied.

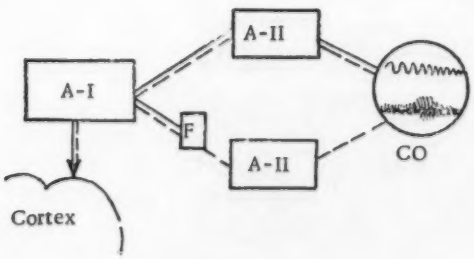

Fig. 1. Over-all diagram of apparatus. A-I – Wide-band amplifier; F – capacitative filter; A-II – amplifiers in cathode-ray oscillograph; CO – recording part of cathode-ray oscillograph (solid line shows path of low-frequency electroencephalographic signal, broken line shows path of high-frequency impulse signal).

It must be noted that there was a considerable difference between the amplitudes of the electroencephalographic and impulse signals: the magnitude of the former was 200-500 μv, while the magnitude of the latter, with this method of recording, was 10-20-50 μv. Therefore, the two signals were finally amplified to different extents by means of the amplifiers in the cathode-ray oscillograph. Figure 1 shows the over-all plan of the method described.

The essence of the method, therefore, is that, out of the whole gamut of frequencies of the incoming signal, we selectively amplified the high-frequency signal, which is smallest in magnitude and represents the total impulse activity of the nerve cells lying near the recording surface of the electrode, and we recorded this high-frequency signal. The low-frequency (electroencephalographic) signal was recorded from the same point with the same electrode; but the low-frequency component has a greater amplitude, so that at this amplification it was clearly displayed, while the impulse component, having a voltage only one-tenth to one-fifteenth as great, was practically indetectible. It is true, however, that in individual instances it was possible to observe some superposition of the impulse signal on the low-frequency component.

This method does not permit us to estimate the activity of individual neurons. It does allow us to characterize the impulse activity, and the degree of synchronization of the impulse activity, of a group of neurons in the vicinity of the recording electrode.

The experiments were performed on unanesthetized rabbits with chronically implanted electrodes, and in acute experiments on rabbits under urethan anesthesia. In all, 37 experiments were carried out.

When the hindleg of the rabbit was stimulated, the following changes in electroencephalographic and impulse activity were observed: in the sensorimotor cortex, more or less "synchronized" electroencephalographic activity gave way to "desynchronization." Against the background of desynchronization, 1-2 seconds after it appears, intense impulse activity developed, continuing for from one to five seconds and generally ending before the desynchronized electroencephalogram returned to its initial state (Fig. 2a). It is readily seen from the oscillogram shown here that the appearance of high-frequency activity is reflected in the electroence-

phalogram in the form of small-amplitude, high-frequency variations; if the electroencephalogram is recorded on an ink-writing electroencephalograph, these variations may be scarcely noticeable, or take the form of high-frequency variations (with a mean period of 0.01-0.02 sec) of insignificant amplitude, superimposed on the electroencephalogram.

As an example of another form of electroencephalographic activity, we have investigated the so-called "4-6 per second" regular rhythm. This rhythm, as is well known from the work of P. K. Anokhin [1] and N. I. Shumilina [4, 5], appears in the parietal and temporal lobes of the cortex and in the reticular formation of the brain stem and thalamus of the rabbit upon the application of conditioned defense stimuli. M. M. Bantsekina [2] has shown that this rhythm can arise in response to a single electrical stimulation of the rabbit's paw. We used this method to obtain the regular rhythm. As may be seen in Fig. 2b, in response to stimulation of the paw, the regular-rhythm reaction appeared in the reticular formation of the rabbit. 0.5-2 seconds after the electroencephalogram changes in a specific manner, a marked intensification of the impulse activity of the cells was noted, which continued for 3-12 seconds.

From these observations it follows that in response to stimulation, the electroencephalogram changes first, with a subsequent change (in our observations, an intensification) in the impulse activity of the nerve cells. The intensification of impulse activity comes to an end before the initial level is restored in the electroencephalogram. These observations show that there can be variations in the relations between the slow electrical activity – i.e., the ordinary electroencephalogram – and impulse activity.

It is clear, however, that the high activity of cellular elements in the reticular formation requires the regular slow rhythm of electrotonic variations. In desynchronization and regular rhythm we have two different forms of electroencephalographic activity, but the impulse activity is the same in the two cases. This naturally leads to the following conclusion: impulse activity cannot produce the electroencephalogram, i.e., the bioelectrical activity of the brain in the frequency range 0.3-100 cps, although it should be noted that the highest-frequency component of the electroencephalogram (with an average period of 0.01-0.02 seconds) can, to some extent, result from impulse activity of nerve cells. But the ordinary electroencephalogram, in all probability, is the reflection of electrotonic processes occurring independently, to some extent, in the brain, although they arise under the influence of the same stimuli.

SUMMARY

The author describes a method for recording both the electroencephalogram and the summed impulse activity from the same point on the cerebral cortex. As established by experiments on rabbits, a change in the EEG always precedes clear-cut changes in the summed impulse activity. Intensification of the impulse activity was observed against a background of desynchronization and against a background of "regular rhythm." This leads to the conclusion that the EEG is not the result of

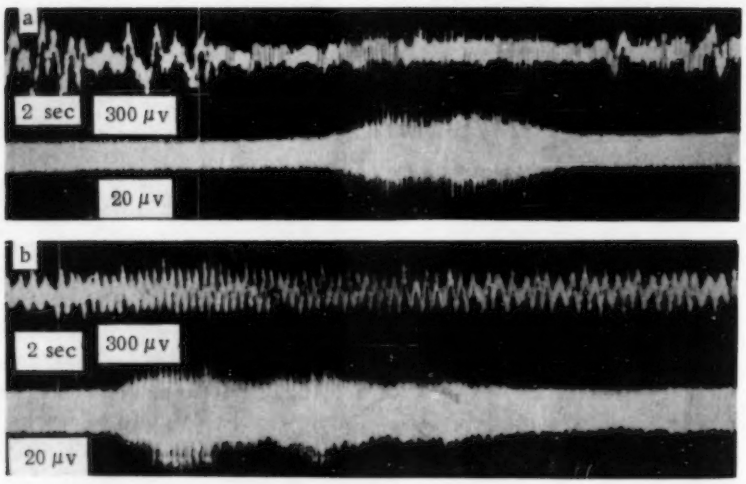

Fig. 2. Oscillogram demonstrating the correlation between the electroencephalogram (upper beam) and the impulse activity (lower beam). a) During the period of desynchronization; b) during period of "regular rhythm." Vertical line on oscillogram shows the moment when stimulus was applied.

algebraic summation of the impulse activity of a large number of nerve cells.

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THE EFFECT ON VASCULAR INTEROCEPTOR REFLEXES OF ANODIC AND CATHODIC POLARIZATION OF THE MEDULLA OBLONGATA

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(Presented by Active Member of the AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 13-19, August, 1960
Original article submitted October 14, 1959

In our studies of interoceptor reflexes in different pathological conditions, we have assumed that the changes found depend to a certain degree on central functional changes, and in particular, on alterations in the excitability of the vasomotor center [9, 10].

In the present work we have studied the action of a direct current on the vasomotor medullary center in order to study changes in the interoceptor reflexes as related to the functional condition of the center.

METHOD

Acute experiments were performed on cats to study pressor reflexes following stimulation of the mechanoceptors of the stomach and urinary bladder. In some experiments, we studied blood pressure changes resulting from stimulation of the pressor region of the medulla. This region usually occupied the cranial third of the medulla, a result which agrees with results published by R. Alexander [15]. The electrodes were applied to the dorsal surface of the brain, lateral to the median sulcus of the rhomboid fossa on each side.

Part of the occipital bone was removed, and some of the cerebellum either excised or else pushed aside by means of a special spatula. The current was provided and controlled by storage cells or dry cells (BAS), a switch, wiper-arm, a resistance box, and a rheostat, which was used for gradually switching the current on or off; the current and voltage were measured by meters.

In the first ten experiments, the skin was not damaged, and the medulla was not exposed; the active electrode consisted of a chlorided cylinder 3 or 5 mm in diameter containing a nonpolarizable paste; it was attached to the skin over the occipital foramen. In the second set of 42 experiments, a nonpolarizable brush electrode ($Zn-ZnSO_4$) was used, and, according to the experiment, was placed at various positions between the middle of the rhomboid fossa and the obex.

When studying the "central pressor response," which develops when the pressor region is stimulated with an intermittent current from a stimulator, direct current was applied to the extreme caudal area of the brain. In the first set of experiments, the indifferent electrode was a plate measuring 4 × 3 cm, covered with silver chloride, and in the second, it was a zinc disk measuring 2.5 cm in diameter; it was fixed by a rubber band placed over a piece of gauze moistened in Ringer's solution beneath the jaw, or sometimes over the shoulder region. In the first set of experiments, the current varied from 0.5 to 100 ma, and in the second, from 0.025 to 16 ma.

The traces given below of the most typical experiments, performed with the direct current values reported in the text, are those of the second set of experiments.

RESULTS

In both sets of experiments, a marked phasic change in the interoceptor reflexes occurred when either the positive or the negative pole of a direct current supply was applied to the medulla.

As can be seen from Fig. 1, when the anode was first applied at a current of 0.2-0.6 ma, the pressor reflexes due to stimulation of the stomach and urinary bladder were increased (traces 3-7). Subsequently, the interoceptor reflexes were definitely suppressed at a current value of 0.8 ma, the effect being particularly marked at currents of from 3 to 5 ma (traces 10-15). The suppression was maintained for 20 minutes after the polarization was removed (trace 16, recorded after a 5 minute interval).

With these current values, a similar phasic response occurred in the "central pressor reaction." It can be seen from Fig. 2 that, when anodic stimulation is ap-

Effect on Interoceptor Reflexes and the "Central Pressor Reaction" of Cathodic Stimulation of the Medulla at a Current of 0.5-3.5 ma (Experiment of May 13, 1958)

Experimental procedure	Time from beginning of the procedure (in minutes)	Duration of cathodic stimulation (in minutes)	Magnitude of pressor reaction (in mm of mercury)		Remarks
			when pressure region of medulla was stimulated at 50 cps, 10 v, 0.05 msec	when pressure in bladder raised to 40 mm of mercury	
Initial conditions	–	–	23	–	–
	–	–	–	9	–
	–	–	–	8	–
	–	–	23	–	–
Cathodic stimulation of 0.5 ma	4	4*	–	19	
Cathodic stimulation of 2.5 ma	1	23*	34	–	
	4	26	–	17	First phase
	7	29	26	–	
	10	32	–	5	
	14	36	22	–	
Cathodic stimulation of 3.0 ma	4	42**	–	Pressor 1 Depressor 1****	
	7	45	18	–	
	13	51	11	–	Second phase
Cathodic stimulation of 3.5 ma	1	53***	–	1	
	4	56	7	–	
After switching off polarizing current	2	–	No reaction	–	–
	5	–	13	–	–
	9	–	–	Depressor 3****	–
	23	–	17	–	–
	28	–	–	8	–
	33	–	17	–	

*Before cathodic stimulation at 2.5 ma, cathodic current was applied for 11 minutes at 0.5 ma, and for 7 minutes at 1 ma.

**[14], p. 349.

*** A cathodic current of 3 ma had been applied previously for 1 minute.

**** Pressor-depressor change of blood pressure.

plied to the medulla for 22 minutes at 0.5 ma or 1 minute at 0.8 ma, there is a marked increase in the response to stimulating the pressor region of the medulla by a 25cps alternating potential of 10 v (traces 5-7). When the polarizing current is increased to 0.8 ma or to 3 ma, which causes a very marked effect, the "central pressor reaction" to the same stimulus is first reduced, and then completely suppressed (traces 8-10). After the constant current had been switched off for a few minutes, there was no response to the stimulus (trace 11), and not until 24 minutes after did a feebly shown pressor response develop interceptor reflexes.

The same phasic change in interoceptor reflexes, as well as the "central pressor reaction," was observed when cathodic stimulation was applied. The following experiment will serve as an example (see table). It

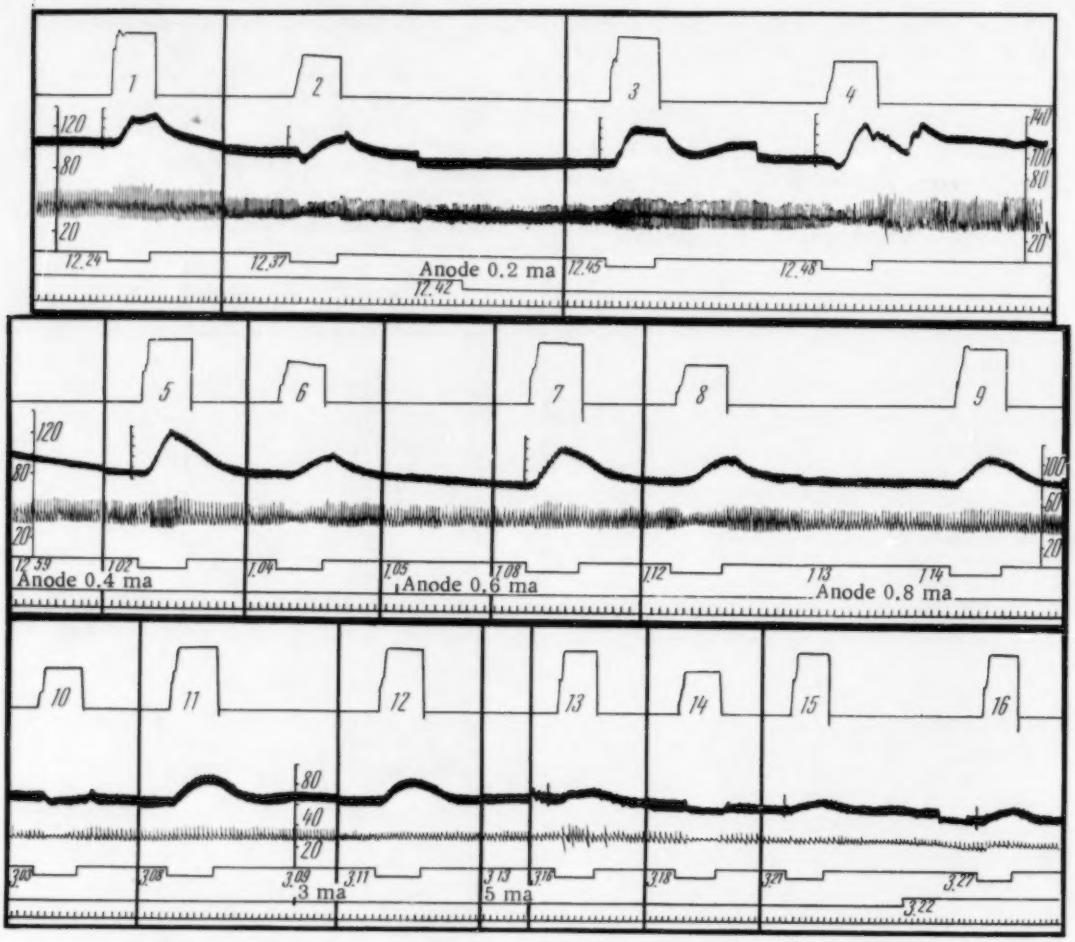

Fig. 1. Biphasic change of interoceptor reflexes in response to anodic stimulation of medulla at a current of 0.2-5 ma. Experiment of Mar. 29, 1958: 1, 3, 5, 7, 9, 11, 12, 13, 15, 16 – reflexes obtained in response to increasing pressure in urinary bladder to 60 mm of mercury; 2, 4, 6, 8, 10, 14 – reflexes in response to increasing pressure in stomach to 40 mm of mercury. Curves, from above downward: Pressure in organ; arterial pressure; respiration; zero line of arterial pressure (also stimulus marker); application of polarizing current; 5-sec time-marker.

can be seen that the first phase, consisting of an initial increase, and the second, comprising the subsequent suppression of interoceptor reflexes, took place at the same time as the corresponding change in the excitability of the part of the medulla which mediates the pressor response.

From the results obtained it follows that when the polarizing current is applied to the medulla, the phasic change in the interoceptor reflexes depends on an alteration of the condition of the central part of the reflex arc. The effects of anodic and cathodic stimulation are approximately the same, the difference being only in the relative strength of the action: Phasic changes of the interoceptor reflexes and their suppression developed more rapidly after cathodic,than after anodic, stimulation. For certain current values, it was, therefore, possible to observe a difference between the action of positive and negative poles. However, since the cathode reduces interoceptor reflexes, when it was replaced by an anode,an increased pressor response and a return of the reflexes previously suppressed by application of the cathode could once more be observed (Fig. 3; compare traces 6 and 7 with trace 9).

However, the restoration of the anodic action on the reflex excitability of the medulla during recovery from cathodic depression took place only for those values of current for which the anode itself exerted no depressive effect. For instance, after reflex depression had

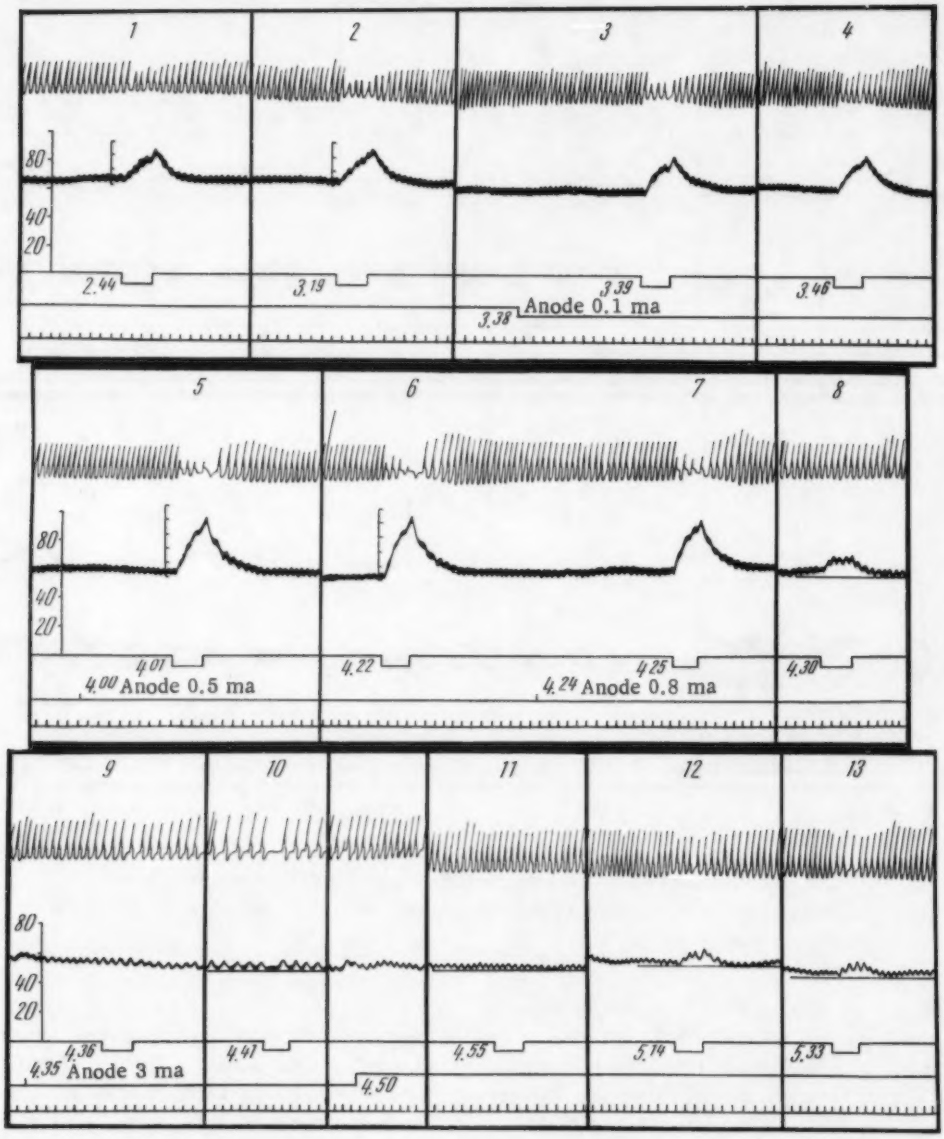

Fig. 2. Biphasic change of the "central pressor reaction" following the application to the medulla of direct current from an anode (0.1-3 ma). Experiment of Apr. 26, 1958. Curves, from above downward; respiration; arterial pressure (this line also represents stimulation of the pressor region of the medulla at 25 cps 10 v 0.05 m sec); 5-sec time-marker.

been induced by cathodic treatment, when using a current of 12 ma, it was not possible to restore interoceptor reflexes by application of the anode, although when polarization was withdrawn, the reflexes once more appeared, and gradually returned to their initial level.

It must also be added that the phasic changes in the interoceptor reflexes, which we have just described as following the application of either cathode or anode to the medulla, do not occur if strong currents equal to the higher values used are applied instantaneously. When this was done, both poles caused an immediate fall and subsequent elimination both of interoceptor reflexes and of the "central pressor reaction."

Since the time that Pflüger's law was published (1859), it has been usual to suppose that the cathode in-

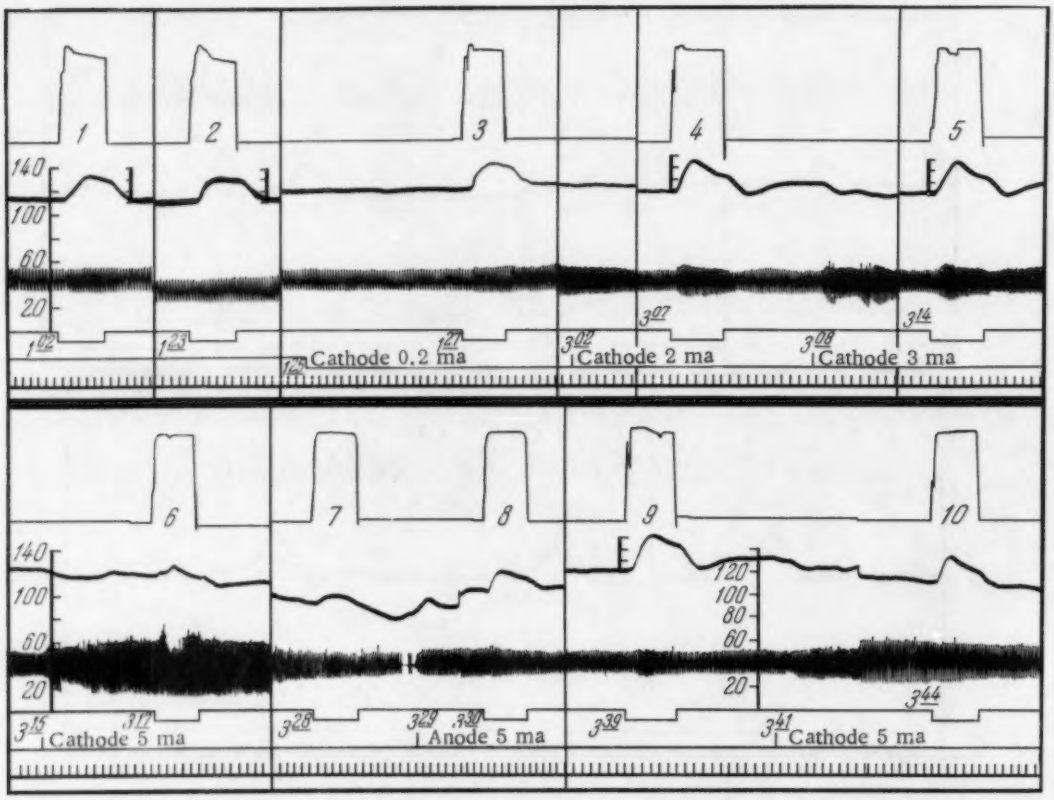

Fig. 3. Restoration of the effect of anodic stimulation with a constant current of 5 ma on interoceptive reflexes during the development of cathodic depression (0.2-5 ma). Reflexes recorded during increase of pressure in bladder to 80 mm of mercury. Curves as in Fig. 1.

creases excitability of nervous structures, while the anode depresses it. The results we have obtained do not fit into this framework of ideas. It must be emphasized that many authors have pointed out the error in Pflüger's conclusions. B. F. Werigo [3] confirmed that cathodic depression follows immediately after increase of excitability by a cathode, and later [4] he stated that "cat-electrotonus causes a gradual reduction in excitability which follows the course of the polarization,"* whereas, in the case of the anode, in all probability the excitability is raised.

B. F. Werigo saw that the reason for his disagreement with Pflüger was that the latter had not taken into consideration either the summation of the branches of the cathodic polarizing and stimulating currents or the reduction of the anodic test current where the ascending direct current is in the opposite direction to the stimulating current (it is known that an intermittent current exerts a stimulating action only at the point of exit from the nerve). Werigo's conclusions were confirmed by B. I. Khodorov, who found that "true changes in the excitability of the nerve at the poles, as our experiments have shown, were the reverse of those reported by Pflüger." † Werigo and Khodorov's refutation of Pflüger's statements has been discussed in detail by D. N. Nasonov [8], who has shown the necessity for reconsidering the classic assumptions regarding the action of positive or negative poles.

Pflüger's explanation of the reverse changes of excitability of nerves after cessation of a constant current has also been criticized, on the grounds that he confused the time of recovery of the initial level of polarization with the duration of postcathodic depression [16]. We must also note reports in which emphasis has been placed on phasic excitability changes following anodic or cathodic stimulation of nervous structures [2, 5, 6, 7, 12, 14]. In experiments by N. V. Golikov and V. L. Merkulov [5], and by B. P. Ushakov and his co-workers [12], in which small currents were applied at extremely short time intervals, there was found to be a similar-

*[4], p. 213.

†[14], p. 349.

ity in the phasic excitability changes following the action of either an electrotonus or catelectrotonus on the nerve.

The absence of any qualitative difference between the action of anode and cathode under certain conditions has also been pointed out by other authors [1, 13, 18]. There are many reports that the cathode has a stronger effect than the anode [5, 7, 11, 12, 17]; for this reason all cathodic phasic changes take place more quickly and at smaller current strengths than do those produced by the positive pole [5, 12, and others]. According to N. V. Golikov and V. L. Merkulov, this clear-cut quantitative difference between the action of the positive and negative poles has been the reason why several authors have concluded that the actions were opposed, and that the cathode exerted a parabiotic, and the anode, an antiparabiotic effect.

The results which we have obtained agree entirely with those of authors who observed the same type of phasic change of excitability in response to the action of either pole, the effect from the cathode being stronger. It must be noted that, in our experiments on the interoceptor reflexes, there could be no summation of the polarizing and the stimulating currents, because the stimulus used was that of a reflex. The similarity in the changes in the interoceptor reflexes and the "central pressor reaction" indicates that experimental conditions were such that there was no physical reaction between the constant current and the stimulus applied to the medulla. The same conclusion is indicated by the fact that in our experiments restoration of the "central pressor reaction" and interoceptor reflexes always took place gradually. In the first moments after switching off the constant current, these reactions, as a rule, remain the same as at the end of polarization.

The course of the changes of the interoceptor reflexes which followed alteration in the functional condition of the central portion of the reflex arc is similar to those which we have described previously in certain general pathological conditions [10].

SUMMARY

Acute preparations of cats were used for a study of interoceptor reflexes involving blood pressure changes, and of the pressor reaction which developed in response to polarization of the medulla oblongata. It was found that both cathode and anode elicited essentially similar phasic blood pressure changes. Because of its relatively weaker effect, the anode restored reflexes (within certain limits) which had been depressed by previous cathodic stimulation.

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THE EFFECT OF MECHANORECEPTORS OF THE URINARY BLADDER UPON THE STRIATED MUSCULATURE

Report I. The Effect of Mechanoreceptors of the Urinary Bladder on the Striated Musculature Taking Part in the Act of Micturition

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 19-24, August, 1960
Original article submitted August 14, 1959

In previous reports [2, 3, 4] it was shown that impulsation in the afferent nerves of the urinary bladder gives rise to certain reflex changes in the tonus of its musculature and the musculature of the urethra, which differ during the filling of the urinary bladder and during its evacuation. It was established that this difference in the reactions is determined by a differing intensity in the afferent impulsation [1, 12]. A number of investigators, having studied the reflex changes during the act of micturition, also noted involvement of a definite group of skeletal muscles in this act.

Thus, D. Denny-Brown and E. Robertson [9], S. Muellner [13] et al., observed contraction of the abdominal muscles during the act of micturition. J. Evans [10] recorded inhibition of the efferent impulses in the nerves innervating the m. levator ani, m. bulbocavernosus, and the muscle of the external urethral sphincter. In addition, many investigators noted relaxation of the external sphincter, which is composed of striated muscle [7, 8, 9, 11, et al.].

In this work we report the results of the investigation, whose purpose was to study the reaction of the external sphincter and the oblique abdominal muscle during the filling of the urinary bladder and at the time of its evacuation.

EXPERIMENTAL METHOD

The experiments were carried out on cats, kept under urethan or nembutal narcosis. The narcotic was injected intravenously. In all, 33 experiments were set up. The action potential of the muscles was recorded by using a double-channeled cathode oscillograph with an amplifier; the monitored band of the latter was set at rectilinear, within the bounds of 10 to 500 cps. For the conduction of the action potentials of the muscles, we used needle-shaped silver electrodes. Stimulation of the mechanoreceptors in the urinary bladder was carried out by filling the bladder with air under a determined pressure. The latter was recorded on a smoked kymograph drum, on which a record was also made of respiratory movements.

EXPERIMENTAL RESULTS

During the time that the urinary bladder remained empty, we noted the background activity of the muscles of the external sphincter as well as the abdominal muscles (Fig. 1a; Fig. 2a). It was shown that, with stimulation of the mechanoreceptors in the urinary bladder, the lowest threshold is observed for the reflex giving rise to alterations in the muscular activity of the external sphincter; in association with this, an increase occurs in the bioelectrical activity of these muscles, at the same time that the activity of the oblique muscles of the abdomen remains the same (see Fig. 1b). As has been shown earlier [2, 4], at this time the impulsation in the n. pudendus, innervating the urethra, becomes stronger, and the tonus of the latter increases.

A small increment in the strength of stimulation of the urinary bladder mechanoreceptors continued, as before, to cause an increase in the biopotentials of the muscles of the external sphincter, while the activity of the abdominal muscles was somewhat inhibited (see Fig. 1c). Further intensification of the stimulation gave rise to rhythmic changes in the bioelectric activity of the muscles of the external sphincter, analogous to the strengthening and weakening periods of efferent impulsation to the urethra via the n. pudendus; at this time the activity of the abdominal muscles continued to be inhibited (see Fig. 1d). Subsequent increase in the strength of stimulation caused inhibition of the activity

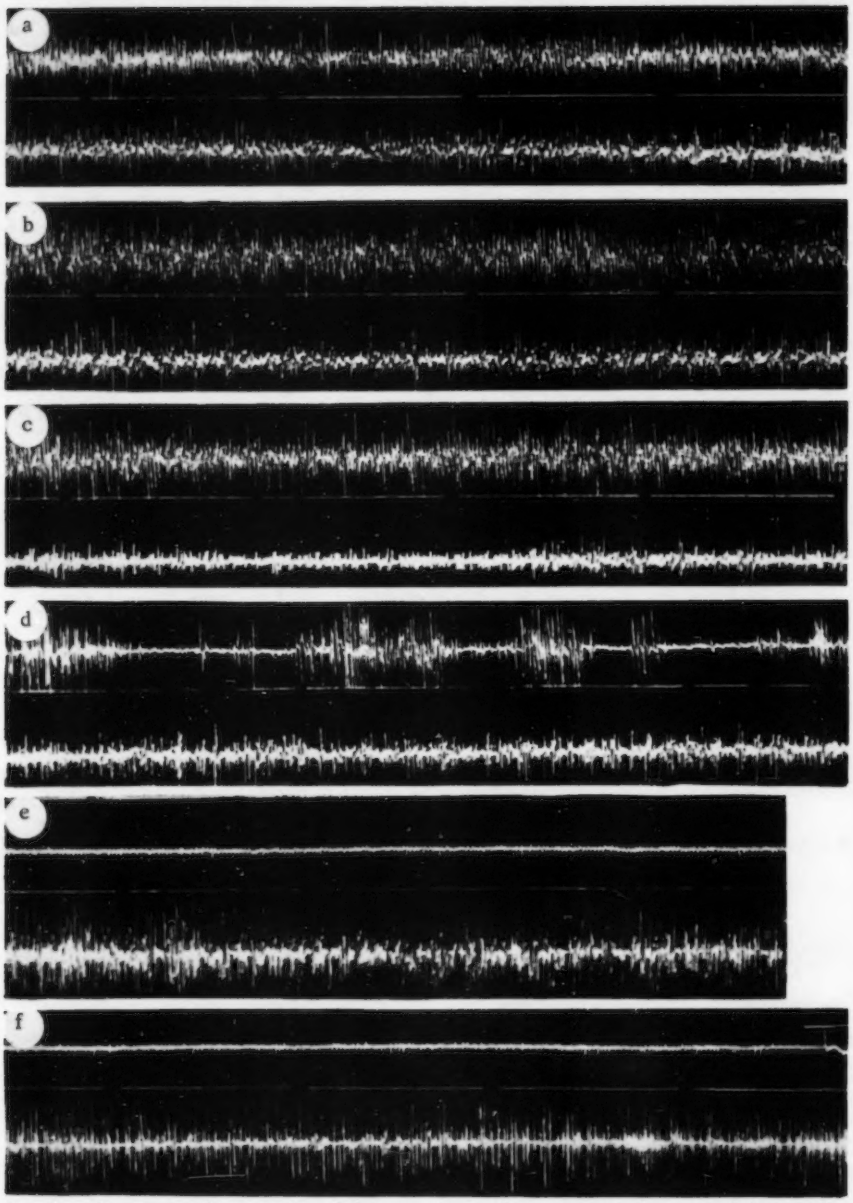

Fig. 1. Changes in the activity of the muscles in the external sphincter of the urethra and the oblique abdominal muscle with varying strengths of stimulation to the mechanoreceptors of the urinary bladder. a) Background activity of the muscles of the external sphincter and the oblique abdominal muscle with the urinary bladder empty; b) pressure in the urinary bladder: 8 mm of mercury, showing that the activity of the external sphincter is increased, and the pressure of the abdominal muscles is unchanged; c) pressure in the urinary bladder: 11 mm of mercury, showing an increase in the activity of the external sphincter and inhibition of the abdominal muscles; d) pressure in the urinary bladder: 18 mm of mercury, showing rhythmic activity of the external sphincter and inhibition of the activity of the abdominal muscles; e) pressure in the urinary bladder: 40 mm of mercury, showing inhibition of the activity of the external sphincter and strengthening of the abdominal muscles; f) pressure in the urinary bladder: 146 mm of mercury, showing inhibition of the activity of the external sphincter and strengthening of the abdominal muscles, but less than in Fig. 1e. Significance of the curves (from above downward): action potentials of the muscles of the external sphincter, time marks – 1 second, action potentials of the oblique abdominal muscle.

in the external sphincter. At this time inhibition of the action potentials in the n. pudendus, innervating the urethra, and relaxation of the urethra, were observed [2, 4]. Simultaneously with the deceleration of the activity in the external sphincter, the activity of the abdominal muscles increased (see Fig. 1e). Contraction became increasingly stronger with subsequent increase in the distension of the urinary bladder. However, extreme stimulation (elevation of the pressure in the urinary bladder to 150 mm of mercury or higher), on the other hand, caused smaller contractions of the abdominal muscles (see Fig. 1f) than stimulation of a more moderate strength. An effect such as this from extreme stimulation was observed in approximately half of the total number of trials; in the other trials, under these conditions, the result was an inhibition in the activity of the oblique abdominal muscles.

In several trials the background activity of the abdominal muscles presented itself as volleys of impulses, synchronous with the respiratory movements. In these trials, as well as in those described above, inhibition was noted in the activity of the abdominal muscles upon weak stimulation of the urinary bladder mechanoreceptors, and an increase in activity was observed in response to strong stimulation, while this increase was less marked with the application of extremely strong stimulation. The reactions of the oblique abdominal muscles often did not coincide with the reflex changes in the respiratory movements, which were inhibited in association with weak and moderate stimulation of the urinary bladder mechanoreceptors, and showed increased strength only with rather intense stimulation of the latter.

In the process of recording the bioelectric activity of the oblique abdominal muscles at their opposite ends (the distal end was placed in direct proximity to the urinary bladder, the proximal end was fastened to the ribs and removed from the urinary bladder for a certain distance), it was possible to observe that the reflex alterations initially appeared in the distal portion of the abdominal muscle and then, after 2-0.3 seconds, they arose in the proximal portion of the same muscle (see Fig. 2a, c). With stimulations of higher strength this difference in the latent periods became smaller or disappeared. Thus, at the strengths of stimulation which act under natural conditions, the reaction of the abdominal muscle begins at that portion of it which is located in the vicinity of the urinary bladder, and only subsequently does the reaction spread into the other portions

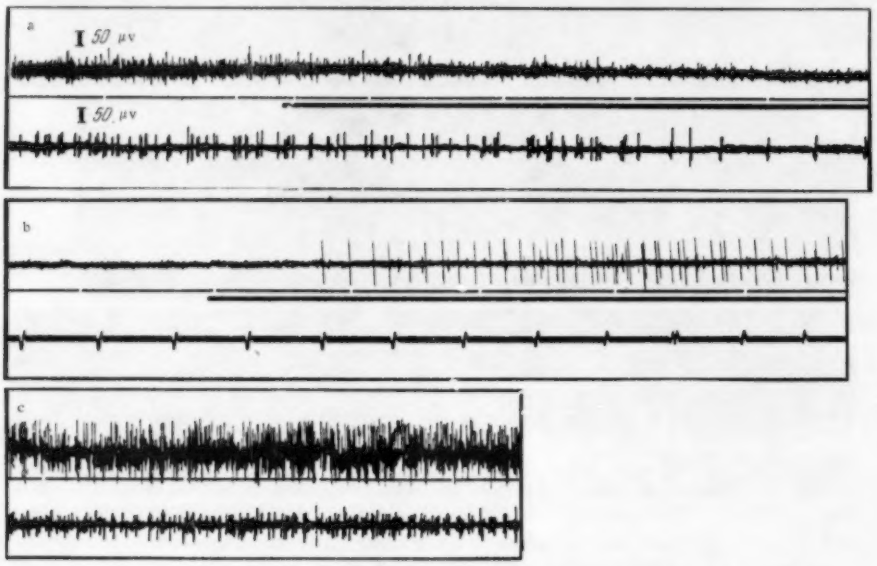

Fig. 2. Changes in the activity of the oblique abdominal muscle at different levels with stimulation of the urinary bladder mechanoreceptors. a) Pressure in the urinary bladder: 16 mm of mercury, showing inhibition of the activity of the oblique abdominal muscle in its distal portion with a latent period of 1 second, in its proximal portion with a latent period of 2.5 seconds; b) pressure in the urinary bladder: 24 mm of mercury, showing an increase in the activity of the oblique abdominal muscle in its distal portion with a latent period of 0.6 seconds, in its proximal portion with a latent period of 4.6 seconds; c) pressure in the urinary bladder: 33 mm of mercury, showing a differing degree of increase in the activity within the distal and proximal portions of the oblique abdominal muscle. Significance of the curves (from above downward): action potentials of the distal portion of the abdominal muscle, time marks – 1 second, stimulation record, action potentials of the proximal portion of the abdominal muscle.

of the muscle. In addition to the indicated difference in the latent periods for the two portions of the oblique abdominal muscle, it was also possible to note a difference in the strength of the reaction of these portions in response to the identical strength of stimulation to the urinary bladder mechanoreceptors. The reaction of the distal portion of the muscle was more intense than the response of the proximal portion (see Fig. 2c).

Thus, the picture described earlier [2, 4] of the reflex changes arising in the period during which the urinary bladder is filling with urine and is subsequently evacuated may be supplemented with the characteristics of the reaction of the striated musculature which takes part in these processes (Fig. 3). It has been established that, in the period when a contraction arises in the muscles of the external sphincter and a relaxation occurs in the muscles of the urinary bladder [4], a relaxation also begins in the abdominal muscles (the threshold of these reflexes is somewhat higher than for the first two). The latter, obviously, leads to a decrease in the intra-abdominal pressure, which engenders an increase in the storage capacity of the urinary bladder. Thus, the conjunction of reflexes in the smooth muscles of the urinary blaader, the striated muscles of the external sphincter, and the abdominal muscles, secures the reservoir function of the urinary bladder.

With an increase in the filling of the urinary bladder, there follows a relaxation of the external sphincter and a contraction of the muscles of the urinary bladder. In

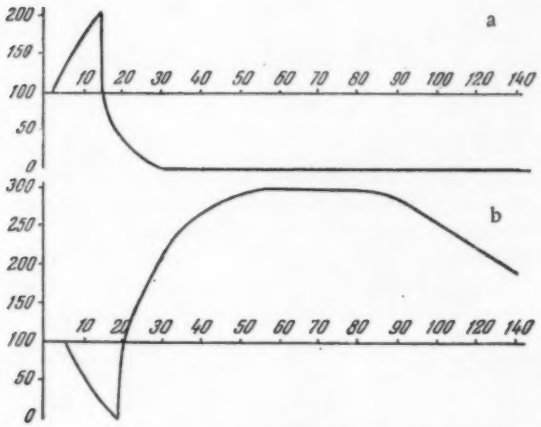

Fig. 3. The relation of the reflex alteration in the activity of the muscles in the external sphincter and the oblique abdominal muscle to the intensity of stimulation of the urinary bladder mechanoreceptors (schematically). On the abscissa – intensity of stimulation of the urinary bladder mechanoreceptors, in millimeters of mercury; on the ordinate – action potentials (a– external sphincter, b– oblique abdominal muscle) in percent of the original level, which was taken to be 100.

those instances wherein a simultaneous contraction of the muscles of the external sphincter and the urinary bladder is observed, the abdominal muscles either still remain relaxed or begin to contract. Contraction of the abdominal muscles becomes more intense when the external sphincter dilates. This reaction promotes the elevation of the pressure in the abdominal cavity and in the urinary bladder, i.e., the most rapid and complete evacuation of the bladder.

Comparing the material of the present report with the data published by us earlier on the reflexes arising from stimulation of the urinary bladder mechanoreceptors [2, 3, 4], we must above all, devote attention to the fact that the range of local or systemic [5, 6] reflexes for the given stimuli is not limited to reflex effects on internal organs, with their characteristic smooth musculature. The external sphincter of the urethra, although indeed consisting of striated muscle, cannot be regarded as skeletal muscle, but the oblique abdominal muscle incontrovertibly belongs to this category. The reflex reactions of these two striated muscles arise with rather low thresholds of stimulation, close to the threshold for the generation of afferent impulsation from the receptors of the urinary bladder, and they undergo changes in functional significance with stepwise intensification of the stimuli, i.e., with transition from a reservoir function for the urinary bladder to an evacuatory one. According to all these characteristics, the reflexes described in this report enter the "physiological system," arising in response to stimulation of the urinary bladder, and they must be included in the "systemic" category. This especially pertains to the reflexes of the external sphincter, appearing in this physiological system as the "most systemic," and, to a lesser degree, to the reflexes of the oblique abdominal muscle, arising from somewhat higher thresholds. The latter may be regarded as less local than the reactions of the renal vessels [3]. In conclusion, it should be noted that, with stimulation of the urinary bladder receptors, the reactions of many internal organs not directly related to urinary excretion must be characterized as much further interlinked than the reactions of the skeletal muscle entering into this physiological system.

SUMMARY

The reflex changes of the activity of striated muscles of external sphincter and oblique abdominal muscle were studied in acute experiments on cats. With low filling of the urinary bladder, the activity of external sphincter (contraction) and the inhibition of abdominal muscles activity (relaxation) becomes increased. This promotes the filling of the urinary bladder, i.e., the accomplishment of its function as a reservoir. With greater accumulation of liquid in the urinary bladder, the activity of the external sphincter is inhibited (it relaxes, and there is a rise of the ab-

dominal muscles' contraction activity), promoting evacuation of the urinary bladder.

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ON THE ADAPTATION OF CHEMORECEPTORS

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 24-28, August, 1960
Original article submitted August 14, 1959

E. D. Adrian [9] first established the fact that various receptors adapt to a constant stimulus. Following him, most investigators limited their study of the phenomena of adaptation to processes occurring directly in the receptor itself [10, 11, etc.]. It was assumed that cessation of reflex reactions during prolonged stimulation results from adaptation of receptors to stimulation. A different tack was adopted by investigators [5, 7, 8] who suggested that in the intact organism adaptation can not be restricted to the receptors, but arises in the analyzer as a whole.

In studying the phenomena of adaptation in the whole organism, A. M. Ugolev, V. M. Khayutin, and V. N. Chernigovskii [7] showed that, in response to prolonged stimulation of the mechanoreceptors of the urinary bladder and intestine, the circulatory and respiratory reflexes, which develop first, disappear although the stimulus continues to act. Analyzing the cause of the adaptation of these reflexes, these authors adduced convincing proof that reflex adaptation was caused by the development of inhibition in the centers. The most conclusive data in this connection are those of V. M. Khayutin [8], who showed in experiments with the cold block of an intestinal nerve during prolonged stimulation of interoceptors that, at a time when the blood pressure had returned to normal, adaptation of the interoceptors still had not taken place, since removal of the cold block produced reflex changes in blood pressure of the same magnitude as at the beginning of the experiment.

Similar results were obtained by T. S. Lagutina [5]. Comparison of the reflex changes in blood pressure with the pattern of afferent impulses during prolonged stimulation of the mechanoreceptors of the urinary bladder, showed that the blood pressure returned to the original level while afferent impulses were still continuing to reach the centers.

It was of interest to determine whether similar interrelationships hold between central mechanisms and the activity of receptors during prolonged application of a chemical stimulus to the receptors.

METHOD

The investigation was performed in acute experiments on cats under urethan anesthesia. The experiments were carried out with perfusion of an isolated segment of intestine having only nervous connections with the organism. The chemical stimuli were injected into the current of perfusing fluid with a syringe for single applications. Prolonged stimulation of receptors was produced with acetylcholine in a concentration of $1 \cdot 10^{-4}$ g/ml by replacing the perfusion fluid (Ringer–Locke solution saturated with oxygen) with the acetylcholine solution. We recorded the pattern of afferent impulses in the intestinal nerves and kept a simultaneous record of the arterial pressure in the carotid artery (with a mercury manometer) on the kymograph. This method has been described in detail in an earlier paper [2].

RESULTS

When 1 ml of acetylcholine solution (in a concentration of $1 \cdot 10^{-4}$ g/ml) was injected with a syringe into the current of fluid perfusing the vessels of the isolated intestine retaining only nervous connections with the organism, reflex elevation of blood pressure occurred, which usually lasted 30-60 seconds (Fig. 1).

With prolonged and continuous stimulation of the interoceptors by acetylcholine at the same concentration, there likewise develops an elevation of the blood pressure at first, which gradually returns to the initial level despite continued stimulation (see Fig. 1b). We will call the time required for this return to normal the "reflex adaptation time," following A. M. Ugolev, V. M. Khayutin, and V. N. Chernigovskii. The reflex effects on arterial pressure that occur upon continuous stimulation of receptors adapt at different rates (requiring from a few tenths of a second to a few minutes) in different experiments, and even during the same experiment. But attention should be paid to the fact that in most experiments reflex adaptation developed within 2-4 minutes, and in some cases, the duration of the reflex

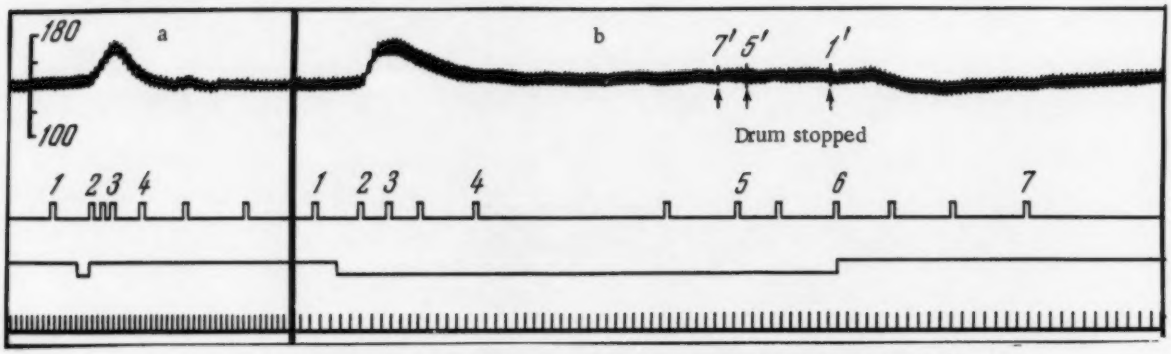

Fig. 1. Reflex changes in arterial pressure accompanying injection of 1 ml of acetylcholine at a concentration of $1 \cdot 10^{-4}$ g/ml into the vessels of the intestine (a) and prolonged passage of acetylcholine in the same concentration through the intestinal vessels (b). Interpretation of curves (from top): recording of blood pressure in the carotid artery; indication of times when oscillograph records were made; injection of acetylcholine; time (5 seconds).

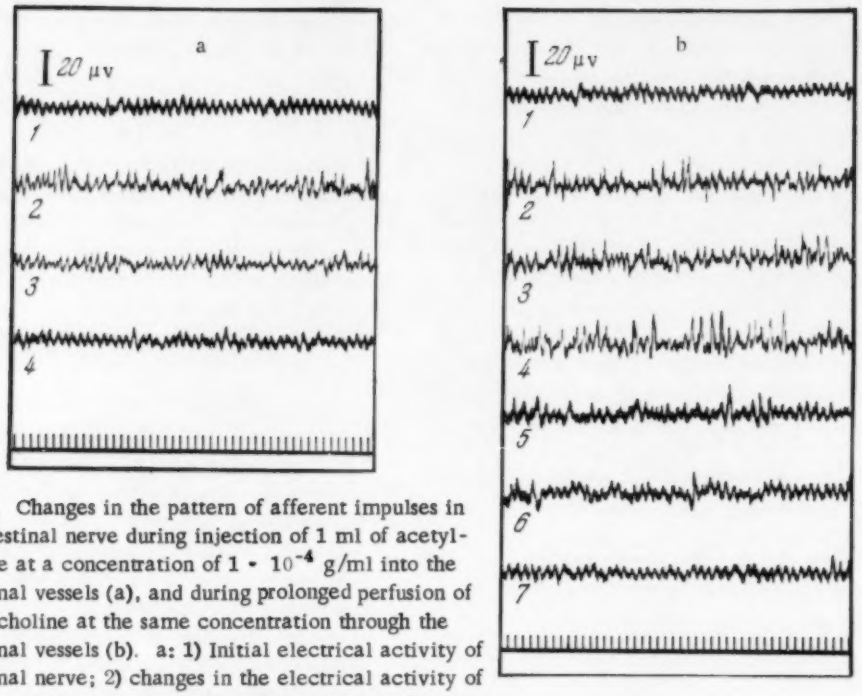

Fig. 2. Changes in the pattern of afferent impulses in an intestinal nerve during injection of 1 ml of acetylcholine at a concentration of $1 \cdot 10^{-4}$ g/ml into the intestinal vessels (a), and during prolonged perfusion of acetylcholine at the same concentration through the intestinal vessels (b). a: 1) Initial electrical activity of intestinal nerve; 2) changes in the electrical activity of the intestinal nerve six seconds after injection of acetylcholine; 3) the same, 20 seconds after injection; 4) the same, 45 seconds after injection; b: 1) initial electrical activity of intestinal nerve; 2) changes in electrical activity of intestinal nerve 10 seconds after beginning of acetylcholine perfusion; 3) the same, 20 seconds after start; 4) the same 65 seconds after start; 5) the same, 10 minutes after start; 6) the same, 17 minutes after start; 7) the same, 1 min 40 sec after start of Ringer-Locke perfusion (time marker – 0.02 sec).

shift in blood pressure accompanying continuous stimulation of receptors was even the same as the duration of the blood pressure shift following a single injection of acetylcholine. In such instances the blood pressure curves sometimes duplicated each other exactly.

Similar variations in the adaptation time of reflex changes in arterial pressure were observed in work cited above [7, 8] with prolonged stimulation of mechanoreceptors of the intestine and urinary bladder. But in our experiments we never observed reflexes lasting for multiples of ten minutes. The longest reflex adaptation time was seven minutes.

When the reflex reaches the level of adaptation, stopping the stimulation of the receptors by switching the perfusion current to pure Ringer-Locke solution produces a drop in arterial pressure to a point below its initial level. This depressor phase is a "release" phenomenon like that observed by A. M. Ugolev, V. M. Khayutin, and V. N. Chernigovskii in their experiments [7]. They interpreted the release phenomenon as a manifestation of inhibition developing in the vasomotor center during prolonged stimulation of the receptors, and clearly exhibited at the cessation of stimulation.

Electrophysiological analysis of the signals from the intestinal receptors during continuous stimulation of those receptors with acetylcholine was carried out by us at the same time as the analysis of the reflex reactions, and showed that during recording of afferent impulses in the intestinal nerves, in response to the injection of 1 ml of acetylcholine in a concentration of $1 \cdot 10^{-4}$ g/ml, there develops a relatively short volley of impulses which usually ends within one minute (Fig. 2a). With prolonged perfusion of acetylcholine solution through the intestinal vessels, the development of the volley of impulses is quite markedly drawn out (see Fig. 2b).

The oscillograms shown in Fig. 2, a and b, were recorded in the same experiment and during the same stimulations as were the kymograms in Fig. 1, a and b. The numerals 1, 2, 3, and so on, on the kymograph records indicate the times when the impulse patterns represented on the oscillograms under the corresponding numerals were recorded. It can be seen from the oscillograms in this experiment that the number of impulses per unit time continues to increase for a considerable period of time (oscillograms 2, 3, and 4), and then diminishes somewhat and remains at a significant level for a rather long time (oscillogram 5); after this, it usually disappears permanently within about half an hour after the beginning of prolonged stimulation.

In the experiment presented in these figures we followed the complete adaptation of the reflex reaction of arterial pressure, with its subsequent "release" at the cessation of stimulation. But we did not observe complete "adaptation" of the receptors, since impulses still kept coming from the receptors (see Fig. 2b, oscillogram 6). This was done intentionally, because, in those experiments where complete adaptation of the receptors was attained during prolonged application of acetylcholine, we failed to observe the "release" phenomenon, which apparently can only appear if there is an abrupt reduction in the rate at which impulses reach the centers.

Thus, electrophysiological investigation of the intestinal receptors during prolonged stimulation shows that these receptors belong in the slowly adapting category. This conclusion agrees with data on the rate of adaptation of other interoceptors. Available information on this subject mainly concerns the receptors of the urinary bladder, which are classified by all authors [1, 3, 4, 10, 11] as slowly adapting.

Comparison of the adaptation time of the reflex shifts in arterial pressure with the adaptation time of the intestinal receptors under prolonged stimulation shows that, when arterial pressure has almost completely returned to the initial level, the rate of impulse generation by the receptors not only has not diminished, but is in fact continuing to increase (see Fig. 2b, oscillogram 4); at a time when the reflex displacement of arterial pressure has adapted completely, the production of impulses remains for a long time at a significant level, adequate to cause a shift in arterial pressure – i.e., the return of reflexes to normal is much faster than the adaptation of receptors.

On the basis of these data we conclude that the return of altered blood pressure to normal during continuous chemical stimulation of intestinal receptors (adaptation to stimulation) is not determined by receptor adaptation, but is apparently the result of the same process of central inhibition whose dominant role in the phenomena of adaptation of interoceptive reflexes has been noted by the authors cited above.

SUMMARY

The subject of this study was the interrelationship between central mechanisms and receptors in the adaptation of reflex blood pressure changes during prolonged action of a chemical stimulus upon the intestinal receptors. The conclusion is drawn that under these conditions the adaptation of the reflex arterial pressure changes is not the result of receptor adaptation, but is mainly due to the involvement of central mechanisms.

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EXTRASPINAL PATHWAYS IN THE REFLEX INTERACTION OF LARGE AND SMALL INTESTINES

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 28-32, August, 1960
Original article submitted July 14, 1959

In the physiological literature and in the clinic, numerous data have been amassed concerning the presence of marked interoceptive effects, not only on neighboring internal organs, but also on distant ones. The mechanism of these effects is still poorly understood. In a number of papers it has been shown that they can be produced within the peripheral divisions of the sympathetic nervous system after destruction of the spinal cord [1, 4, 13, 15, 16, 17, 18, 20, 21, and 22].

In studying the interneuronal connections of the organs of the abdominal cavity, we [10] established that in man and the rabbit the two mesenteric nervous plexuses that innervate the small and large intestine are connected by a short cut by means of the intermesenteric tract. This is located to the left, and partially in front of, the abdominal aorta and is connected with the inferior mesenteric ganglion. These data and experiments on rabbits [6, 9] have shown that, except for monkeys [3], the tract is the most differentiated in these animals, and as was supposed [2], it contains ascending and descending fibers, which allows us to regard it as a complex of interorgan connections, creating the functional interaction between the caudal division of the large intestine and the small intestine that has been demonstrated by a number of authors [8, 11, 12, 14].

In the present investigation we undertook the further study of the significance of the intermesenteric tract in this interaction.

METHOD

The work was carried out on 23 rabbits. In rabbits immobilized by high spinal cord transection, the abdominal cavity was opened. A loop of jejunum was placed in a glass cylinder with no bottom, which was held firmly in an incision in the anterior abdominal wall and filled with Ringer's solution at 38°. The movements of the intestine were recorded on kymograph paper. From the caudal portion of the large intestine, a segment 8-10 cm long was separated with its mesentery. A rubber balloon was inserted into it that could be inflated with air to obtain a pressure of 80-200 mm Hg. During the experiments or ten days previously, the caudal mesenteric ganglion was decentralized by severing all its nervous connections with the marginal sympathetic trunks and the abdominal aortic plexus. The hypogastric nerve, which connects this ganglion with the pelvic nervous plexus, was sectioned at this time, and in some experiments the spinal cord was destroyed below the tenth thoracic segment. Diplacine was used to block synaptic connections in the ganglion. After this the investigation proceeded.

RESULTS

In the initial background, rhythmic contractions of the jejunum occurred at a frequency of 12-18 waves per minute, and slight variations in tone were noted. Upon mechanical stimulation of the caudal portion of the large intestine against a background of intensive movements of the small intestine, inhibition of these movements and a reduction in tone occurred (Fig. 1a); if a segment of small intestine was in a state of comparative rest, mechanical stimulation of the large intestine produced no perceptible changes in the kymograph tracing.

In addition, the intensity of stimulation of the large intestine and the rate of increase of stimulus intensity were important: an intense mechanical stimulus (200 mm Hg instead of 100 mm), or a rapid inflation of the balloon, caused more pronounced inhibition of the peristaltic movements of the small intestine. These changes ordinarily were observed at the moment of stimulation of the large intestine; aftereffects were not seen.

Decentralization of the caudal mesenteric ganglion, either 10 days in advance or at the time of the experiment, resulted in enhancement of the movements of the small intestine: the rhythm of contractions was intensified, the frequency of the waves increased to 20 per minute, and unusual variations in tone appeared. Under

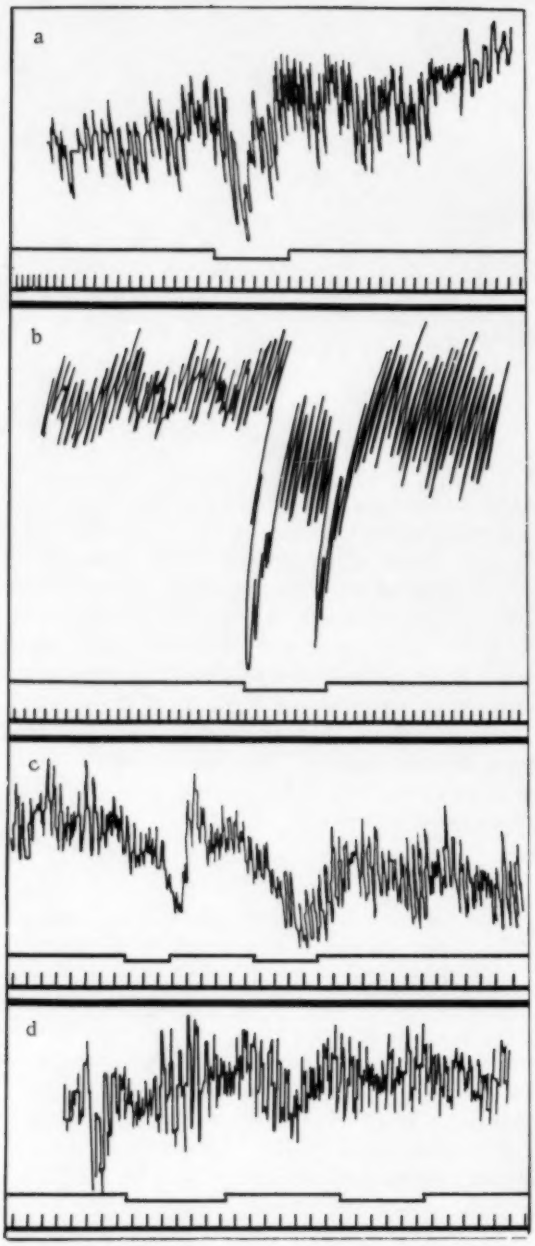

Fig. 1. Changes in interoceptive afferent effects exerted on the small intestine by the caudal portion of the large intestine in the rabbit. a) Initial background (rabbit No. 22, experiment of December 28, 1958); b) after decentralization of caudal mesenteric ganglion (rabbit No. 18, experiment of June 16, 1958); c) after destruction of the spinal cord (rabbit No. 11, experiment of December 27, 1957); d) after section of the intermesenteric tract (rabbit No. 11, experiment of December 27, 1957). Interpretation of curves (from top): movements of small intestine; mechanical stimulation of large intestine; time (5 sec).

these conditions mechanical stimulation of the segment of large intestine continued to inhibit the small intestinal movements, with this effect even being enhanced at times (Fig. 1b), and being observed even after destruction of the spinal cord (Fig. 1c). Sectioning of the intermesenteric tract performed against this background caused a considerable reduction in the inhibition of motor activity of the small intestine, or complete absence of such inhibition (Fig. 1d), and in only two experiments was it as pronounced as in the initial state.

The data obtained confirm the presence of a reflex interconnection, not only between adjacent segments of the gastrointestinal tract [16, 22], but even between such distant segments as the initial portion of the small intestine and the caudal portion of the large intestine. On the basis of the fact that these interorgan interoceptive effects are predominantly of a neuroreflex nature [11, 14, 22], we sought to determine the specific pathways by which they are mediated.

Analysis of the distribution of the interneuronal connections of these organs in the rabbit (Fig. 2) allowed us to suggest three main pathways of afferentation from the portion of the large intestine under investigation: 1) along its intramural nervous plexuses and the arciform connections of the mesenteric nerves accompanying the vascular arcades, in the cranial direction; 2) along the mesenteric nerves to the caudal mesenteric ganglion, and then along its connections with the sympathetic trunks, through the corresponding spinal ganglia into the spinal cord, from which, along pre- and postganglionic neurons within the celiac and cranial mesenteric plexuses, impulses arrive at the small intestine; 3) along the mesenteric nerves to the caudal mesenteric ganglion, and from there along the intermesenteric tract connected with it, directly to the small intestine, within the cranial mesenteric plexus.

The possibility that these interoceptive effects were spreading toward the pelvic plexus and out from it, or along the first of the pathways indicated above, was excluded, since the balloon was introduced into a segment of large intestine that was isolated from its proximal and distal portions. In order to interrupt transmission along the second pathway, formed by afferent spinal fibers, an anatomically thorough decentralization of the caudal mesenteric ganglion was carried out under the magnifying glass, and in some experiments, destruction of the spinal cord as well. Under these conditions the most probable pathway for reflex interaction of the isolated fragment of large intestine with the small intestine was the third, the shortest and most direct extraspinal pathway, represented by the intermesenteric tract (12, 14, and 15, Fig. 2).

The fact that after decentralization of the caudal mesenteric ganglion even more intense inhibition of peristalsis was observed in the small intestine, and a reduction of its tone, when the caudal portion of the large intestine was stimulated, apparently indicates that

this autonomic ganglion was liberated from inhibitory centrifugal influences from the spinal cord [19, 23, 24]. This type of enhancement of viscerovisceral reflexes was noted by E. I. Sinel'nikov et al. [16] after destruction of the spinal cord. According to M. G. Durmish'yan [4], the spinal cord "imposes," so to speak, definite functional characteristics upon the autonomic ganglia.

Subsequent section of the intermesenteric tract led in most experiments to complete disruption of the reflex connection between the large and small intestine. The same results were obtained with prior decentralization of the ganglion, where, as I. P. Razenkov has shown [13], all the preganglionic effector fibers of spinal origin are found to have degenerated, and the possibility of transmission of stimulation by axon-reflex connections is eliminated. It must be suggested that the transmission of interoceptive influences in our experiments might to some extent be brought about (the two experiments mentioned give evidence of this) along "short pathways," formed by the extremely fine retroperitoneal nerve fibers [7] and vascular nerves which run along the branches of the caudal mesenteric artery to the abdominal aorta and from there to the perivascular plexus of the cranial mesenteric artery.

From histological study of preparations impregnated by the Bielschowsky-Gross method, it was established that after section of the intermesenteric tract a large number of intact fibers remain in its distal portion along with the degenerated ones. In addition, in the ganglia and connections of the celiac and cranial mesenteric plexuses we found many more altered fibers than in the caudal mesenteric ganglion and the plexus surrounding it. Finally, section of the tract results in degeneration of some of the neural elements in the first part of the small intestine and its mesentery, and stimulation of the tract with an induction current produces inhibition of peristalsis of the small intestine [9]. All of this confirms the opinion that predominantly ascending fibers are present in the tract [2, 10, 25]; these fibers, it must be supposed, have a relation to the nerve cells of the ganglion itself or constitute a continuation of those afferent sympathetic connections that enter into the composition of the mesenteric nerves from the caudal portion of the large intestine. These nerves, according to I. F. Ivanov [5], represent the processes of Dogiel's type II

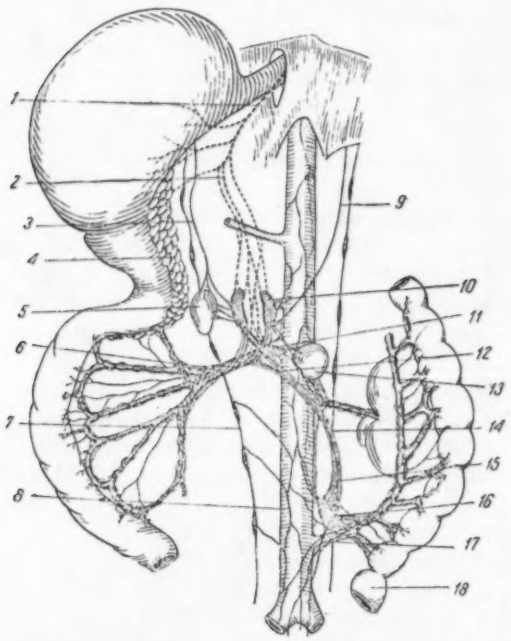

Fig. 2. Interneuronal connections of the caudal portion of the large intestine and the first portion of the small intestine in the rabbit. Semischematic drawing from preparation. 1) Posterior trunk of the vagi; 2) caudal rami of the posterior trunk; 3) right splanchnic nerve; 4) pancreas; 5) right adrenal; 6) cranial mesenteric plexus; 7) sympathetic trunk; 8) abdominal aorta; 9) left splanchnic nerve; 10) left celiac ganglion; 11) cranial mesenteric ganglion; 12) cranial portion of the intermesenteric tract; 13) left adrenal; 14) intermediate portion of the intermesenteric tract; 15) caudal portion of the intermesenteric tract; 16) caudal mesenteric ganglion; 17) caudal mesenteric plexus; 18) caudal portion of the large intestine.

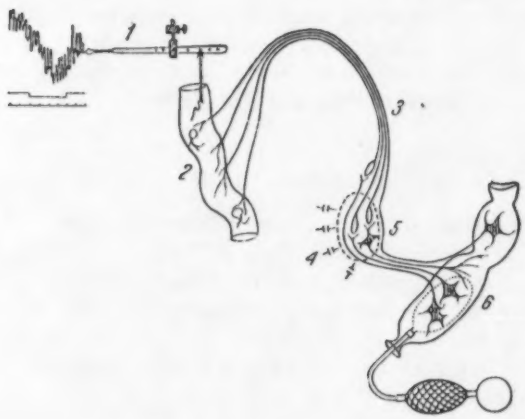

Fig. 3. Diagram of possible interneuronal connections mediating transmission of interoceptive afferent influences from the caudal portion of the large intestine to the small intestine in the rabbit, along the intermesenteric nerve tract. 1) recording of small intestinal movements with an Engelmann lever; 2) loop of jejunum; 3) intermesenteric tract; 4) sites of section of central connections of the ganglion; 5) caudal mesenteric ganglion; 6) caudal portion of the large intestine with balloon inserted into it; sensory cells are dark, motor cells are light.

receptor cells and can be traced to the prevertebral ganglia. According to Kuntz [22], fibers of intestinal origin enter into synaptic connections with motoneurons of the caudal mesenteric ganglion. In addition, it is known [13, 17] that this ganglion also contains sensory cells.

Experiments with blocking of the caudal mesenteric ganglion with diplacine showed that the observed viscerovisceral influences are not completely eliminated. This indicates that some of the afferent intestinal fibers penetrate into the tract without interruption in the ganglion. Finally, the fact that in a number of experiments, the observed reflex remained even after extirpation of the ganglion is explained by the presence of nerve trunks, readily distinguishable in total preparations stained with methylene blue, that enter into the tract from the mesentery without passing through the ganglion. All these possible afferent interneuronal connections are represented in Fig. 3.

The data obtained show that the intermesenteric tract is one of the main autonomic extramedullary pathways along which interoceptive afferent influences from the caudal portion of the large intestine spread to the first portion of the small intestine.

SUMMARY

In rabbits the transmission of inhibitory interoceptive effects from the caudal portion of the large intestine to the first portion of the small intestine takes place mainly along the intermesenteric nerve tract, following decentralization of the caudal mesenteric ganglion. When this nerve tract is divided, in most experiments, no viscerovisceral reflex effects are observed.

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THE STUDY OF ORIENTING REACTIONS OF THE PUPIL TO LIGHT*

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(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 33-37, August, 1960
Original article submitted July 5, 1959

The theory of orienting reflexes has undergone much development in recent years and, thanks to the investigations of E. N. Sokolov [7], has attracted considerable attention among physiologists and clinicians. A highly convenient object for studying orienting reflexes is the pupil of the eye, which is very responsive to any external stimulus.

Special investigations of the duration of the latent period, rate, and amplitude of contraction and dilatation of the pupil, etc., have been undertaken repeatedly for the purpose of utilizing these reactions in delicate functional diagnosis [1, 2, 8, 12]. Substantial difficulties have arisen, however, in connection with methods of recording the movements of the pupil. In this respect, the objective and precise method of cinematic recording of pupillary contractions proposed by A. R. Shakhnovich in 1956 is very useful [9]. Subsequent development of the techniques has led to the creation of the new method of local pupillography [3, 4].

The study of pupillary reflexes with the aid of the method of local pupillography has shown that pupillary contractions observed immediately after illumination of the normal eye have a twofold character. The clear-cut miosis that occurs in response to a strong light stimulus is a species of typical defense reflex. At the same time, the very similar pupillary contractions that follow less intense illumination have features that are more characteristic for reflexes of the orienting type [10]. In themselves these contractions of the pupil are very similar – they have the same latent period (0.2 sec), they take place at approximately the same rate (between 0.5 and 0.7 sec from the beginning of contraction to the attainment of maximum miosis), and display very little difference in amplitude of contraction. These characteristics of the course of reactions of the normal pupil to illumination, which have been studied in detail by Löwenstein [15], are seen distinctly in many of our pupillograms. But the gradual extinction of pupillary reactions to repeated illumination, which Löwenstein calls the phenomenon of "pupillary fatigue," has not been confirmed in the experiments of other investigators [13].

Employing bright lights, we also were unable to demonstrate, with a precise graphical method, any signs of a reduction in the amplitude of pupillary contractions in response to light, even after 100, 200, or more repeated illuminations [5]. At the same time, weaker illuminations (of approximately 0.2 stilbs or less) invariably were accompanied by extinction of the reflex, which set in the more rapidly, the less the intensity of the applied stimulus – the light.

This difference in the reactions to strong and weaker illumination in the presence of a definite end point – the threshold of development of pupillary reflexes that are extinguished and those that are not – permitted us to conclude that we are dealing here with two essentially different types of pupillary reflexes: a defense reflex to strong light, which is not extinguished, and a reflex of the orienting type to less intense illumination, which is extinguished.

The great outward similarity of pupillary contractions of these two types caused us to attempt to construct experiments in which the difference in the character of the pupillary reflex could be demonstrated not only by employing repeated light stimuli, but also directly, at the very first examination. For this purpose we decided to undertake prolonged illuminations of the eye with light of various intensities.

METHOD

Whereas, in giving a brief stimulus, we switch the light on for exactly 0.2 second -- in the expectation that the whole period of stimulation will fall within the latent period of the pupillary reflex, and that by the time the pupil begins to contract, the light will no longer be acting on the eye--for a prolonged stimulus we decided to use a 3-second illumination of the eye. With brief stimulations (lasting 0.2 second), the time elapsing between the moment of switching on the light, and the moment when the whole reaction is over and the original pupillary diameter is restored to normal, is 1.2-1.5 seconds, including the entire latent period, the period of contraction, and the period of dilatation. On the basis of this interval, a duration of prolonged illumination was chosen in our experiments equal to twice

*All experiments were performed in the eye-examining room of the Institute of Neurosurgery, AMN SSSR.

the period of maximum duration of the physiological pupillary reflex to a brief illumination – i.e., three seconds.

In both brief and prolonged illumination, we produced focal illumination of only a small area in the region of the most posterior portion of the retina – in the zone of the macula lutea. As in all previous experiments, we used faint background illumination for taking photographs, with an intensity of about 25 luxes at the level of the eye being examined. For all our photographs the bulbs, shielded with frosted glass, used for background illumination were placed beneath the eye being photographed, considerably below the line of sight. This location is very convenient for taking photographs, since the light from the background lamps falls on the iris at such an oblique angle that almost none passes through the pupil to the retina, whereas the principal immediate object of cinematography – the iris – is illuminated with sufficient intensity. Figure 1 shows a diagram of the arrangement of the object of fixation (from which the eye is illuminated), the mirror used to direct the strongly stopped-down, narrow beam of light through the pupil onto the retina of the eye being examined, and the lamp for background illumination, in relation to the eye of the observer. This arrangement of the background lamps explains the fact that in our experiments the pupil initially has a diameter of 6-7 mm, i.e., it is not much narrower than in complete darkness. If, at the same intensity of background illumination, the subject fixates on the illuminating lamps, the diameter of the pupil immediately decreases to 4.5-5 mm. In a table presented in Schober's book [16], which was taken from Hartinger's work [14], it is found that the mean pupillary diameter at an intensity of illumination of 10 luxes,at the level of the eye being examined,averages 4.97 mm; and at an intensity of 100 luxes, it is only 3.92 mm for the normal human eye.

In all our experiments we have taken motion pictures at a speed of 10 frames a second, which permits us to trace all the individual moments in the course of the pupillary reflex, and at the same time, does not yield curves that are too flat and stretched-out. Each vertical line on our pupillograms represents 0.1 second.

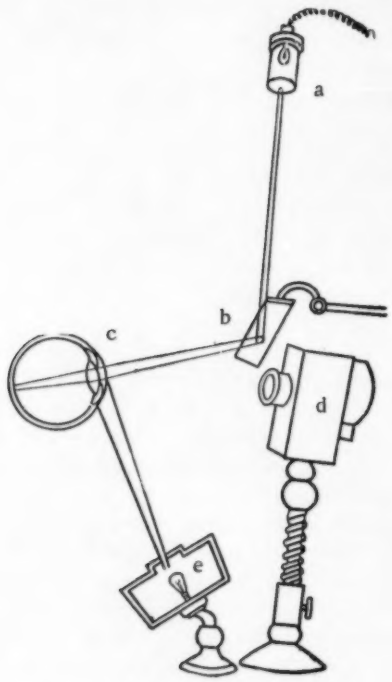

Fig. 1. Sketch of arrangement of illuminating system in local pupillography. a) Fixation object and lamp for illumination; b) mirror used for fixation and illumination of eye; c) eye being examined; d) motion-picture camera; e) lamps for background illumination.

RESULTS

The reactions of the normal human pupil to bright illumination of various durations are shown in the pupillograms depicted in Fig. 2. On the left the figure shows a pupillogram obtained with brief illumination lasting 0.2 second. The pupillogram on the right represents the pupillary reaction to illumination of the same intensity, but lasting three seconds. The initial pupillary diameter of the eye being examined was approximately 6 mm in both cases.

It can be seen from Fig. 2 that after latent periods of the same length in both cases (0.2 second), contraction of the pupil began and took place at roughly the same rate; and that this pupil, with both brief and prolonged illumination,contracted to 4.75 mm in diameter by the 5th frame after the beginning of contraction (the 7th frame from the beginning of illumination), after which it began to dilate again in the case of brief illumination, until,by the 12th frame from the start of illumination,it again returned to its initial diameter.

With prolonged illumination the initial course of pupillary contraction is exactly the same, but contraction of the pupil does not end at the seventh frame – it continues further at the same rate. The pupil does not reach minimum dimensions until the 9th frame after the beginning of illumination, i.e., until a time when,with brief illumination at the same intensity, a considerable degree of pupillary dilatation has already occurred. Corresponding to the longer period of pupillary contraction, its constriction is here more pronounced. In the ninth frame after the beginning of illumination,the pupil has a total diameter of 4.5 mm and remains this narrow as long as the light is applied. After the light is turned off,the pupil remains maximally constricted for another 0.3 second, and then begins to dilate gradually. The process of pupillary dilatation to a diameter of

roughly 5.5 mm occurs at the same rate as with brief illumination, and lasts for 0.5 sec, after which the rate of dilatation slows markedly, so that even by the end of the recording (2 sec after the light is turned off), the pupil still does not entirely reach its initial diameter.

The course of pupillary reaction to weak illumination of different durations is shown in Fig. 3. In this figure, as in the preceding one, the left pupillogram corresponds to a brief illumination lasting 0.2 second. The differences between this and the pupillary reaction to brief stimulation of the eye with light of ten times greater intensity take the form only of somewhat less intense miosis in response to the weaker illumination, but neither the duration of the latent period nor the over-all configuration of the pupillograms shown in Figs. 2 and 3 suggests that the pupillary reflexes to brief illumination of the eye with bright and dim light are different in principle. But clear-cut essential differences in the pupillary reflexes to illumination at high intensity (approximately 2 stilbs) and at low intensity (approximately 0.2 stilb) are immediately apparent when we compare the two pupillograms obtained with prolonged illumination of the eye at the two different intensities.

On the right side in Fig. 3 is shown a pupillogram obtained with prolonged illumination of the eye with a weak light (roughly 0.2 stilb). It is noteworthy that the constriction of the pupil in response to prolonged illumination exactly reiterates the pattern of its reaction to brief illumination at the same intensity. With both prolonged and brief illumination, after a latent period of 0.2 second, a very slow pupillary constriction lasting 0.5 second began, with the pupil constricting from its initial 6 mm to only 5.2 mm. After this period of maximum constriction, with both brief and prolonged illumination a gradual dilatation of the pupil began, taking place somewhat less rapidly than had the constriction. In complete contrast to the reaction to prolonged illumination at high intensity, during prolonged stimulation with a dim light-despite continued application of the light-the pupil again dilated and, in the tenth frame from the beginning of dilatation, again reached its original dimensions. Switching off the light produced no new changes in the diameter of the pupil.

If we compare the pupillograms obtained with prolonged illumination of the eye with bright and dim light, we become convinced of the fact that we are dealing with two reflexes that are essentially different. Stimulation with strong light gives a stable miosis that lasts as long as the application of the stimulus continues, which corresponds to our conception of a pupillary reflex of purely defensive character, directed toward a reduction in the total number of light rays entering the eye and stimulating the light-sensitive elements of the retina. When the amount of light entering the eye is no longer excessive, the pupil again dilates to its previous dimensions.

The pupillary reaction to a dim light proceeds in an entirely different fashion. In this case the pupillary contraction to prolonged illumination takes exactly the same form as the contraction to a brief light of the same intensity, since the novelty of the stimulus is the basis of the reaction. Just as repetition of a series of brief, faint illuminations has the result that the pupil stops reacting to them with constriction, so also, with prolonged illumination at low intensity, the contraction of the pupil caused by the appearance of the light immediately disappears as soon as the influence of the light loses the element of novelty. Thus, the entire course of the pupillary reaction to prolonged illumination at low intensity compels us to regard it as an orienting reflex to something new. From these premises it becomes possible to understand how the reflexes to brief and to prolonged illumination at low intensity are completely alike, and coincide in all respects with pupillary reflexes to changes in the form or color of the fixated object, which have been described in the work of A. R. Shakhnovich [11].

Thus, the use of prolonged illumination allows us to determine immediately whether we are dealing with pupillary contractions of a defensive or an orienting character, and this in turn may have some practical importance for the clinic, e.g., in topical diagnosis of certain forms of brain disease. In a previous paper [6]

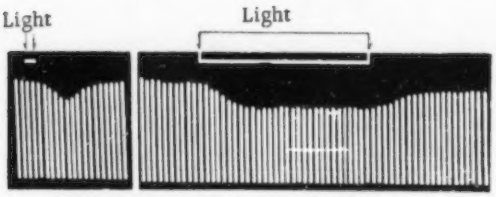

Fig. 2. Contraction of pupil under the influence of a bright light (about 2.0 stilbs). On the left – effect of brief illumination lasting 0.2 sec; on the right – effect of prolonged illumination at the same intensity (lasting 3.0 sec).

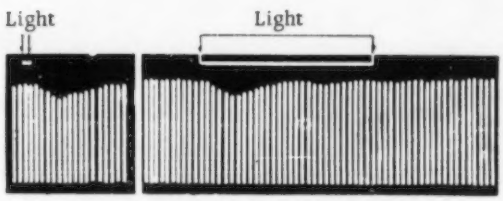

Fig. 3. Contraction of pupil under the influence of a dim light (about 0.2 stilb). On the left – effect of brief illumination (0.2 sec); on the right – effect of prolonged illumination (3.0 sec) at the same intensity.

we noted that, in lesions in the region of the visual funiculi, we observe a loss of pupillary contractions of both the defensive and the orienting character. On the other hand, in cortical lesions only the orienting pupillary reflexes are lost, and not the defense reflexes. The possibility of determining quickly and accurately whether a lesion is a purely basal one in the area of the visual or oculomotor tracts, or whether the patient is suffering from disturbances in the cerebral cortex, can hardly be overrated, especially if we are concerned with comparatively small, localized inflammatory foci that are not manifested in clear-cut neurological symptoms, or the earliest stages of development of brain tumors.

SUMMARY

Pupillary constriction to light may take the form of defense or orienting reflexes. To distinguish the reflexes of these two types, the author suggests prolonged, instead of brief, exposure, to light; in the first case the defense reaction gives a stable miosis lasting throughout the exposure period, whereas the orienting reaction takes the form of a brief constriction of the pupil, which then resumes its former size despite continued exposure to the light.

The possibility of promptly distinguishing defense from orienting reflexes is of practical, as well as theoretical, significance, since loss of the defense reaction of the pupil to light points to a basal localization of cerebral lesions, involving visual or oculomotor pathways; on the other hand, loss of the orienting reflex alone points to a cortical localization of the lesion.

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EXCRETION OF VITAMIN B_6 IN THE URINE OF A HEALTHY INDIVIDUAL

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 37-39, August, 1960
Original article submitted February 15, 1959

From our own and from published observations, it would appear that increasing ascorbic acid intake normally causes an increased amount of it to be excreted in urine collected over a definite period of time in the morning, before food is taken.

The object of the present investigation was to study the effect of an increased percentage of ascorbic acid in the food on the excretion of vitamin B_6, as determined from the amount of 4-pyridoxic acid in the morning urine collected before food was taken.

METHOD

The observations were made on 38 men aged 19-25 years. They lived under the same conditions and took the same food.

During the whole period, they were kept on a standard diet whose constituents were not varied from day to day. The daily ration contained an average of 115 g of protein (including 44 g of animal protein), 79 g of fats, and 614 g of carbohydrates. The calorific value was 3,721 calories. The calculations were made from tables of the chemical composition of the food product [2].

The ascorbic acid content of the prepared food was found to vary from 0.6 to 19.5 mg, and had an average

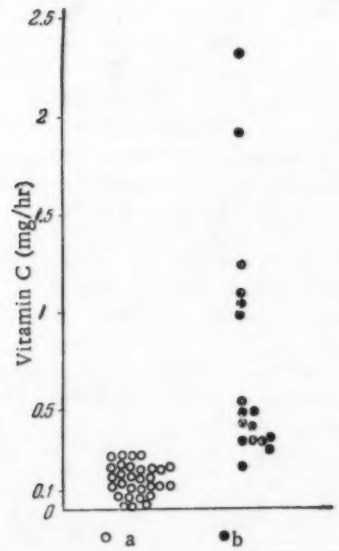

Fig. 1. Excretion of vitamin C in the morning urine before taking food. a) Before vitamin treatment; b) by the 49-51st day after taking 100 mg of ascorbic acid daily.

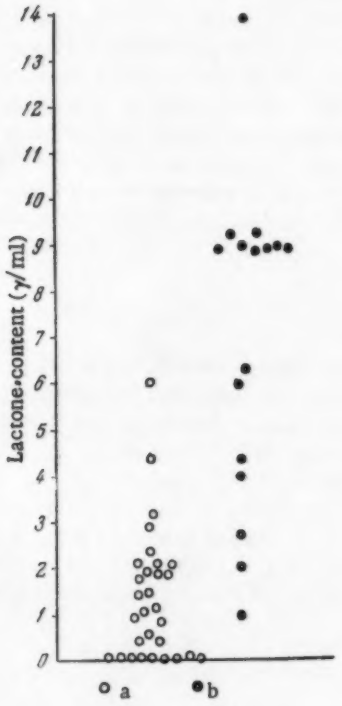

Fig. 2. Amount of the lactone of 4-pyridoxic acid. a) Before taking vitamin C; b) on the 49-51st day after taking 100 mg of ascorbic acid daily.

value of 8.8 ± 0.6 mg per day. Measurements of fruit and vegetable dishes were made by the simplified method using the All-Union State Standard (GOST 7047-55).

The urine was collected at a definite period before food was taken, but after the night urine had been voided. Measurements were made of the amount of ascorbic acid and of the lactone of 4-pyridoxic acid: For the first determination, the urine was titrated in the presence of acetic acid with 2,6-dichlorophenolindophenol and for the second, the fluorometric method [3] of Huff and Perlzweig was used. For this purpose 1 ml of urine was poured into each of two test tubes, and hydrochloric acid was added. One tube was the control, and the other was heated for 15 minutes at 100° in order to obtain the lactone of 4-pyridoxic acid. The contents of both tubes were then diluted, and borax was added to bring the pH to 9.0. A comparison was then made of the fluorescence in both test tubes with that of the standard lactone solution. A Bach lamp and a Wood's filter were used for exciting the fluorescence.

To determine the lactone of 4-pyridoxic acid without adding pyridoxin, V. A. Bogdanova [1] recommends diluting the urine further up to 200 times (and if this is not sufficient, up to 400 times) in order to reduce the fluorescence of impurities which may interfere with the determination of lactone. When we had become familiar with the fluorometric method, we were convinced that, when making large numbers of determinations of the lactone of 4-pyridoxic acid in the urine of subjects who had not received additional vitamin B_6, it was most convenient to use the maximum dilution of 400 times straight away. In many cases this procedure eliminates certain superfluous measures which are unavoidable if the solution has been insufficient. By diluting 400 times at once, we were able to carry out up to 12 determinations per day.

RESULTS

Thirty subjects were kept on a diet low in ascorbic acid, and one test tube from each was tested. The morning urine contained a small amount of ascorbic acid having a range of from 0.03 to 0.29 mg/hr, and a mean value of 0.18 ± 0.01 mg/hr. At the same time we demonstrated the small amount of the lactone of 4-pyridoxic acid whose range of values was 0-6 γ/ml, with a mean value of 1.45 ± 0.23 γ/ml. By calculating the amount of the lactone of 4-pyridoxic acid per portion of urine collected hourly, it was found that the excretion varied from 0 to 161.9 γ/hr, and had a mean value of 40.6 ± 6.9 γ/hr.

Some of the subjects received up to 100 mg ascorbic acid per day as tablets. After 49-51 days, samples of urine from 17 subjects were examined, and it was found that the ascorbic acid excreted in the morning urine ranged from 0.23 to 2.28 mg/hr, with a mean value of 0.72 ± 0.13 mg/hr (Fig. 1). At the same time there was a considerable increase in the amount of the lactone of 4-pyridoxic acid: It varied from 2 to 14 γ/ml, and had a mean value of 6.28 ± 0.74 γ/ml. (Fig. 2). The calculated amount of lactone per portion of morning urine ranged from 66.4 to 456.5 γ/hr, and had a mean value of 234.6 ± 24.4 γ/hr.

The results obtained for the content of 4-pyridoxic acid in human urine agree with those of V. A. Bogdanova who found that, without added vitamin B_6, from 0 to 14γ of lactone per ml of urine was present.

Mathematical treatment of the results which we obtained showed that, for ascorbic acid and for the lactone of 4-pyridoxic acid (in g per ml and in g per hr), the difference between the excretion rates before and after taking the vitamin was statistically significant.

We also discovered a new phenomenon; namely, that the enhanced excretion of ascorbic acid in the urine caused by a raised ascorbic acid intake brings about a spontaneous increase in the excretion of the lactone of 4-pyridoxic acid.

SUMMARY

Morning urine on an empty stomach taken from males aged 19-25 years, fed on a diet deficient in ascorbic acid, was found to contain a small proportion both of this acid and of vitamin B_6, excreted in the form of lactone of 4-pyridoxic acid. When these subjects were given 100 mg of ascorbic acid daily, there was a statistically significant increase in the amount of ascorbic acid and 4-pyridoxic acid excreted in the morning urine.

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FACTORS INTERFERING WITH OXYGENATION OF THE BLOOD IN PNEUMOTHORAX

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No, 8, pp. 40-45, August, 1960
Original article submitted July 10, 1959

We have shown previously [6] that processes which cause respiratory changes (as affecting the general level of pulmonary ventilation, its evenness, and coordination of the respiratory movements), may lead to arterial hypoxia; it was also shown that, of these factors, the principal one was that of impaired respiration. The changes in blood oxygenation were brought about, not only by altered pulmonary ventilation, but by a second important factor– namely, the flow of blood in the lesser circulation.

In the present work we have studied the effect on blood oxygenation of changes in the pulmonary circulation under various conditions. As a method of altering pulmonary ventilation as well as circulation, we used unilateral pneumothorax (open and closed).

Pneumothorax causes a marked discoordination between the ventilation and blood supply to the lungs, and therefore, may appear to be a cause of arterial hypoxia. In fact, with open unilateral pneumothorax, one lung collapses, and ventilation in it ceases entirely. Under these circumstances there is a redistribution of blood in the lesser circulation, and blood from the right ventricle flows through the vessels to the second lung, which continues to function; therefore, the ventilation of this lung may be insufficient to saturate the flow of blood, which has increased twofold [4, 7]. Also, it is not known to what extent the blood flow to the collapsed lung has decreased; this factor must also be of significance in the development of hypoxia.

From their very nature, the phenomena which develop in a closed pneumothorax must differ from those in the open operation: According to modern views, in a closed pneumothorax the lungs continue to perform respiratory movements [2, 8, 9, 10]; however, it is possible that even in a closed pneumothorax the normal relationships between ventilation and pulmonary circulation are disturbed.

It seemed worthwhile to learn how arterial hypoxia occurs in pneumothorax; we therefore attempted to determine the importance both of changes in respiration and in the redistribution of blood in the lesser circulation as affecting the development of hypoxia.

METHOD

The experiments were performed on rabbits under urethan or morphine anesthesia. Rabbits were used because with them it is possible to dispense with artificial respiration, since the two pleural cavities are separate: When the cavity on one side is opened, the lung on the opposite side continues to function; the closed pneumothorax was also made unilaterally. Recordings were made of the respiratory movements, pulmonary ventilation, blood pressure, and arterial blood-oxygen saturation (this was done by means of a photoelectric instrument applied directly to an artery).

RESULTS

The experiments showed that when one pleural cavity was opened, the respiratory movements of the thorax increased; the greatest increase was in the inspiration, indicating an expansion of the thorax (Fig. 1a). Despite the increase in respiratory movements, pulmonary ventilation was reduced, sometimes more than twofold (see Fig. 1a). Thus, while the blood supply to the functional lung was improved, its ventilation did not change, and might even have been reduced. By itself, entry of air into a pleural cavity did not cause any great change in blood pressure (see Fig. 1a); only in cases when opening the cavity was associated with a sufficiently intense mechanical stimulation of the pleura was there any fall in blood pressure (followed by recovery).

To determine whether there were changes in the pulmonary circulation, we used an indirect index, which was the degree of arterial blood-oxygen saturation. The reasoning was that if, when the lung collapses, the blood flow through it is stopped completely, the blood-

oxygen saturation, which will have fallen after effecting the pneumothorax, ought after a time to recover partially (partially, because in the second lung some disturbance of blood oxygenation is to be expected). If part of the blood continues to circulate through the vessels of the collapsed lung, it will become mixed with blood which has passed through the functional member, and in this way a reduced arterial blood-oxygen concentration will be maintained. In this case, when the circulation to the collapsed lung is artificially blocked by clamping the main artery supplying it, it is to be expected that the blood-oxygen saturation will be increased.

Immediately after opening a pleural cavity, there was a marked reduction in the saturation of the blood (see Fig. 1a). For the most part, the saturation was either maintained at this reduced level for a considerable time (until the end of the experiment, i.e., for several hours), or else even continued to fall; only in a few experiments did it recover partially.

When a changeover was made from breathing air to breathing oxygen, arterial saturation increased considerably, but it hardly even reached the level corresponding to that obtaining when oxygen was breathed before the pneumothorax. When the artery supplying the collapsed lung was clamped, there was usually a further increase in arterial oxygen saturation (see Fig. 1b); this result indicated that before clamping, some of the blood continued to pass through the collapsed lung. The phenomenon was observed repeatedly during a single experiment for a considerable time after opening a pleural cavity (for several minutes, and sometimes for several hours after the collapse of the lung). E. M. Kreps and V. I. Voitkevich [3] observed a similar phenomenon during pneumonectomy in human subjects: On ligaturing the pulmonary artery (35 minutes after effecting deep pneumothorax), the saturation of the arterial blood was increased by 10-12 %; this fact can be explained only by supposing that blood from the collapsed lung which had not been oxygenated was pre-

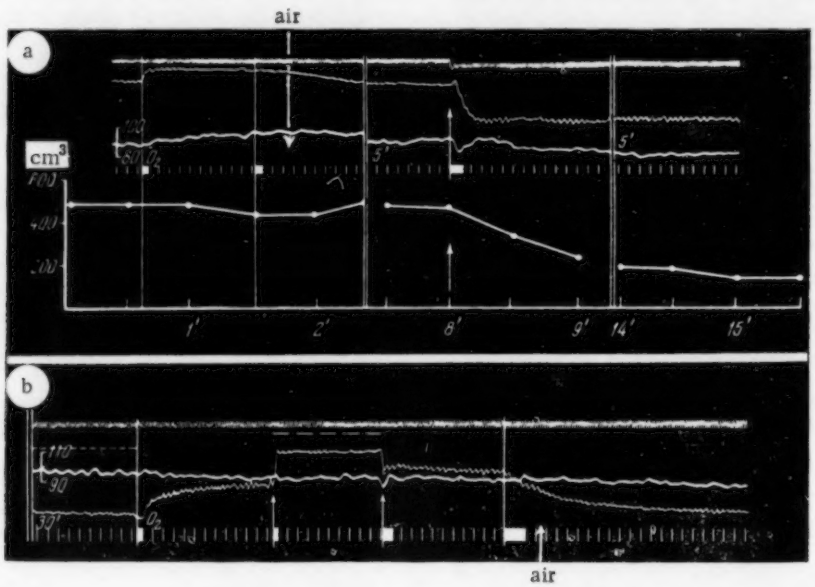

Fig. 1. Changes in the degree of arterial blood-oxygen saturation following open pneumothorax. Experiment of April 14, 1958. (Rabbit weighed 2.75 kg.) a) Response to inspiring oxygen, before pneumothorax (indicated by vertical lines) and to opening of one pleural cavity (shown by arrow). Curves, from above downward: Respiration (pneumogram), degree of arterial blood-oxygen saturation, blood pressure, 5-sec time-marker, pulmonary ventilation (in cm^3 per 30 sec). The two vertical lines indicate a break in the recording, whose duration is shown by the figures alongside; b) response to breathing oxygen after an open pneumothorax (beginning and end of respiration shown by vertical lines) and compression of the main pulmonary artery on the side of the pneumothorax (time of compression of the artery and of removal of the clamp are shown by arrows). Curves, from above downward: Respiration (pneumogram), blood pressure, degree of arterial blood-oxygen saturation. Broken lines indicate level of arterial blood-oxygen saturation before effecting an open pneumothorax: while breathing air - - -; when breathing oxygen ———. 5-sec time-marker.

vented from mixing with the arterial blood from the functional lung. S. L. Libov and V. I. Burakovskii [4], using angiocardiography, observed that the opaque substance was delayed in the collapsed lung; this result indicated a constriction of the pulmonary vessels on the side of the pneumothorax, which reduced the entry of blood, although, as our experiments have shown, the blood flow is not entirely arrested.

To differentiate phenomena originating in the collapsed lung from those which occur in the functional lung, and which also may be the cause of a maintained hypoxia, we have studied the effect on arterial blood saturation of breathing oxygen. When blood flow to the collapsed lung is stopped, and oxygen is breathed, it is to be expected that the blood will be normally oxygenated, because the increased partial pressure of oxygen in the inspired air should compensate for the insufficient ventilation of the remaining lung. Nevertheless, in many cases, even after tying off the one artery and breathing pure oxygen, the arterial circulation did not reach the level established before the pneumothorax had been effected (see Fig. 1b). This result indicates that in the operating lung, blood must pass along paths in which it does not come into contact with the oxygen of the alveolar air, and most probably it flows along arteriovenous anastomoses, so that the alveolar capillaries are short-circuited. We have previously observed this same phenomenon in humans after pneumonectomy [5], where again there was failure to obtain arterial saturation when oxygen was breathed, which indicated that part of the blood was flowing along arteriovenous anastomoses of the lesser circulation.

It might therefore, be supposed that arterial hypoxia following open pneumothorax is due chiefly to two

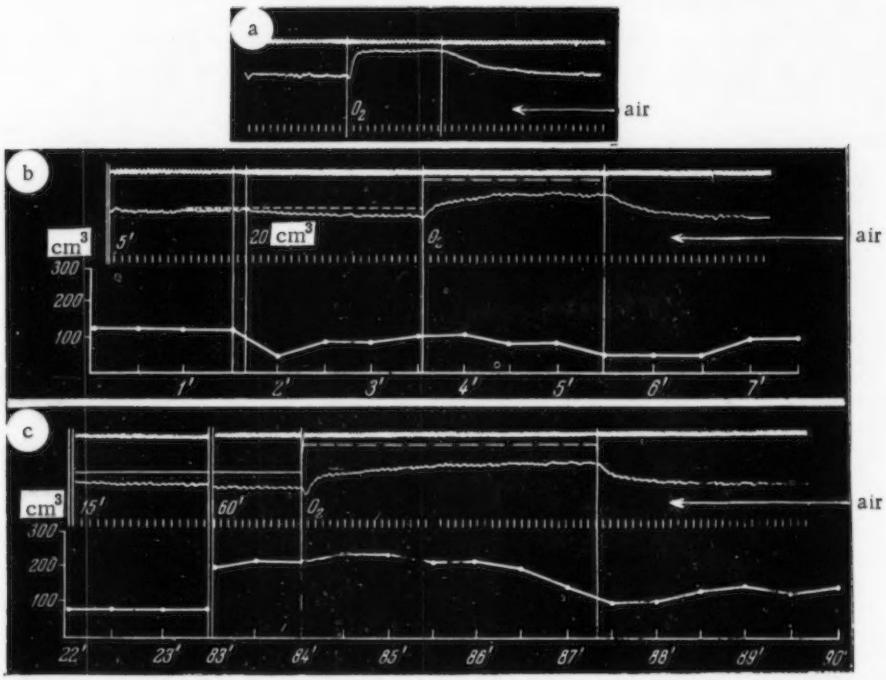

Fig. 2. Changes in the arterial blood-oxygen saturation following closed pneumothorax. Experiment of October 30, 1958. (Rabbit weighing 2.7 kg). a) Response to breathing oxygen before the pneumothorax; b) response to injection of 20 cm^3 of air into the left pleural cavity, and to breathing oxygen 2 minutes after effecting the pneumothorax; c) response to breathing oxygen 1 hour 20 minutes after pneumothorax. Curves, from above downward: For a) respiration (pneumogram), degree of arterial blood-oxygen saturation, 5-sec time-marker; for b) and c) respiration (pneumogram), degree of arterial blood-oxygen saturation, 5-sec time-marker, pulmonary ventilation (in c.c per 30 seconds); for b) the dotted line indicates the degree of arterial blood-oxygen saturation before effecting the closed pneumothorax: while air was breathed (---); when breathing oxygen (— — —). The degree of arterial blood-oxygen saturation before the closed pneumothorax was effected is shown by the continuous horizontal line when air was breathed, and by the line of long dashes when oxygen was breathed.

factors: First, there is a considerable increase in the blood flow to the remaining functional lung, and insufficient compensatory increase in ventilation; secondly, for a considerable time (not less than several tens of minutes or even several hours), blood continues to flow through the collapsed lung without becoming oxygenated. In addition, some of the blood may flow through arteriovenous anastomoses of the functional lung; in this way the resistance of the lesser circulation may be reduced, but the hypoxia then inevitably becomes deeper.

Experiments with closed pneumothorax showed that when 20-40 cm^3 of air are introduced into the pleural cavity, the rate of respiration is increased, and the depth reduced; at the same time there is a marked fall in the arterial blood saturation (Fig. 2b). Usually, after a few minutes the pulmonary ventilation rate recovers or attains a level higher than the original rate. However, the arterial blood-oxygen saturation remains reduced for a considerably longer time, and may remain so until the end of the experiment, i.e., for several hours. When oxygen is breathed, the arterial saturation never attains that maximum level which obtained when oxygen was breathed before the closed pneumothorax was effected (see Fig. 2).

In experiments in which an open pneumothorax was effected, sometimes, in order to let the animal "rest," we closed the opening in the pleura (the open pneumothorax was transformed into a closed one). Subsequently, the following changes could be observed: The respiratory movements immediately became reduced, but the pulmonary ventilation gradually increased, and sometimes attained the level observed before the open pneumothorax had been performed; the arterial blood-oxygen saturation was also increased,

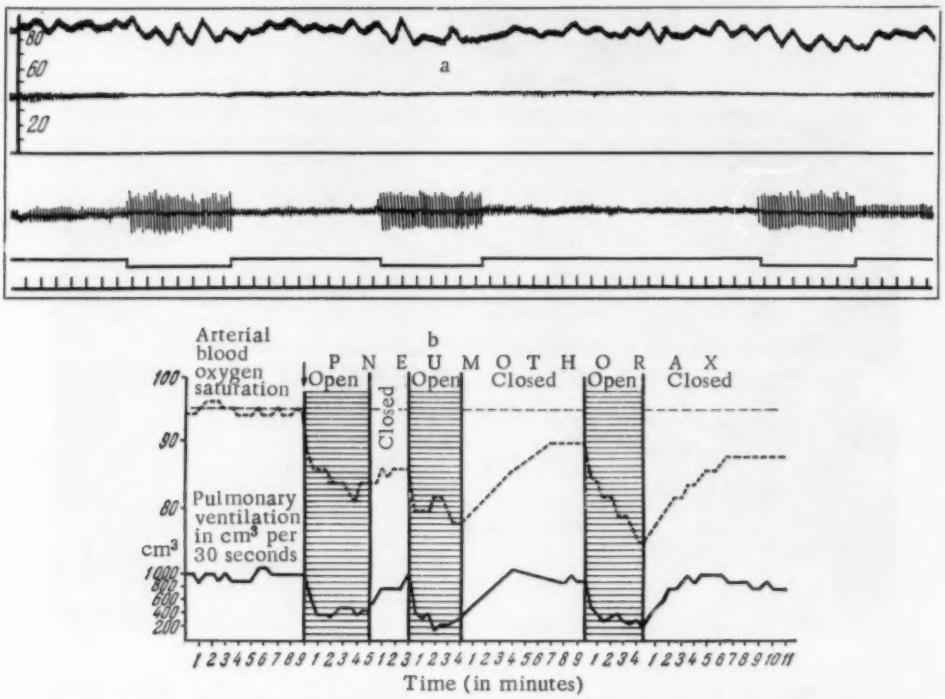

Fig. 3. Effect of hermetically closing the opening in the pleura of an open pneumothorax. Experiment of February 13, 1954. (Rabbit weighed 3.5 kg.) a) Curves, from above downward: Blood pressure, respiration (pneumogram), zero line of manometer, respiration (trace from trachea , stimulus marker (a movement downward corresponds to closing the pneumothorax, and upward to opening it), 5-sec time-marker; b) curves, from above downward: degree of arterial blood-oxygen saturation (in scale divisions of the galvanometer), pulmonary ventilation (in cm^3 per 30 seconds). Shaded columns represent duration of the open pneumothorax. The arrow indicates the moment of opening the pleural cavity. The dotted line represents the level of arterial blood-oxygen saturation before effecting the open pneumothorax.

but remained well below the original level (Fig. 3).

These results indicate that when a closed pneumothorax has been effected, despite the rapid restoration of a normal pulmonary ventilation, the balance between ventilation and pulmonary blood supply is disturbed. It appears that the ventilation on the side of the pneumothorax is greatly reduced. That there is a reduced expansion of the lungs–at any rate, in the first few minutes after producing a closed pneumothorax–has been demonstrated by electrophysiological investigations in which it was found that the number of impulses along the afferent fibers of the vagus was reduced. Nevertheless, even a comparatively small reduction in alveolar ventilation makes the aeration of the normally less ventilated portions insufficient. At the same time, the redistribution of the blood flow in the lungs may not correspond to the altered aeration: Blood flow may continue through the unventilated or poorly ventilated parts of the lungs. Also, as in the case of an open pneumothorax, blood may flow along arteriovenous anastomoses, because, after air has been introduced into the pleural cavity, it is found that the blood is still not saturated even when oxygen is breathed (see Fig. 2).

After a pneumothorax, a considerable time is required for the establishment of normal relationships between pulmonary ventilation and the blood supply which will restore a normal oxygenation of the lungs; it is scarcely possible to observe the development of this condition in an acute experiment.

We may therefore suppose that after an open or closed pneumothorax has been effected, not only disturbed ventilation, but also an impaired lesser circulation is responsible for the abnormal relationships between ventilation and blood supply and for the consequent impaired arterial hypoxia.

SUMMARY

Acute experiments on rabbits under urethan or morphine anesthesia were performed, and the causes of arterial hypoxia following unilateral open and closed pneumothorax studied. It was shown that, in addition to the disturbed pulmonary ventilation, impaired pulmonary circulation also played an important part.

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THE EFFECT OF EXPERIMENTAL NEUROSIS ON AGE VARIATION OF HEART RATE IN NORMAL APES AND IN THOSE SHOWING A BRADYCARDIA WITH SINUS ARRHYTHMIA

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 46-49, August, 1960
Original article submitted October 12, 1959

It has frequently been reported that apes are valuable as experimental animals for studying various pathological human conditions [1, 5, 6, 10]. Cases of coronary insufficiency and myocardial infarct resulting from experimentally induced neuroses have been described [6, 11].

Here we describe a further cardiac disturbance, bradycardia with sinus arrhythmia, also caused by experimentally induced neuroses. Since no reports are available of the normal range of heart rate in apes, we here report the results we have obtained for different age groups.

METHOD AND RESULTS

The heart rate was measured by an electrocardiogram (EKG), recorded by an ÉKP-4 apparatus or by an ink-writing electrocardiograph (Officine Galileo). The neurosis was induced by disturbance of the 24-hour rhythm [10], by altering the daily program at a frequency to which the animal was unable to adapt. We used four 2-year-old hamadryad baboons. (Abad, Omar, Barsuk, and Khisar).

In normal baboons, the heart rate is quite high. Observations on 79 specimens showed no difference between rhesus monkeys and hamadryad baboons, and the results may therefore be taken together. There was, however, a considerable difference according to age (see table and Fig. 1). The differences in heart rate were statistically significant.

It can be seen from the table that the highest rate in the very young animals is 59% above that of the adults; in the one-to-four age group it is lower, and in the adults, lower still. In the old animals, the few observations available indicate that the rate does not differ from that of younger adults.

The marked variation, caused especially by muscular activity and emotional factors, should be noted; it occurs within each group, and the rate for one animal also changes between successive measurements. In the experiments of N. I. Lagutina and A. A. Fufacheva, blocking of the vagi by subcutaneous injection of atropine, or (in an acute experiment) by a ligature, caused an increase in heart rate. However, in apes, the heart rate increases by 17-19% as a result of this procedure, whereas, in man, an atropine injection may increase the rate to 150 beats per minute, which represents a 100% tone is considerably less than in man. This low tone is also indicated by the fact that respiratory arrhythmia is very poorly shown. Figure 1f shows an EKG recorded over a period of 12 seconds when the respiration rate

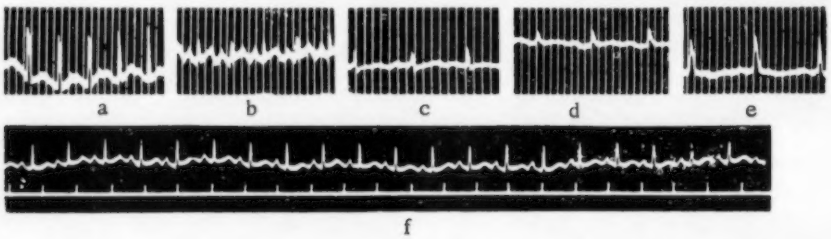

Fig. 1. EKG of apes of different age groups. a, b) 7 months (pulse 300 and 266 beats per minute); c) 2 years (pulse 184 beats per minute); d) 6 years (pulse 150 beats per minute); e) 16 years (pulse 133 beats per minute); f) EKG of adult ape, recorded over a period of 12 seconds (no respiratory arrhythmia).

Range of Variation and Mean Values of Heart Rate in Apes, According to Age Groups

Heart rate	Age			
	less than one year	from one to four years (young)	from four to fifteen years (adult)	from fifteen years (old)
Maximum	300	240	218	171
Minimum	218	133	120	120
Mean	258 ± 4.87 (from 23 measurements)	180 ± 2.89 (from 91 measurements)	162 ± 3.56 (from 35 measurements)	162 ± 2.7 (from 3 measurements)
P	P = 0.0000		P = 0.0007	

was 30-40 per minute. The R–R intervals remain strictly unchanged.

In apes, there is a marked diurnal rhythm in the heart rate. Although the EKG was taken on waking, the conditioned reflex association with night-time causes a reduced rate by night. For adults, a rate below 120 per minute represents bradycardia, and above 240, tachycardia.

In the four animals in which neurosis was induced, the original rate was within the normal limits for the age group: In Barsuk it was 170 per minute, in Khisar 170-190, in Abad 155-170, and in Omar it reached the upper normal limit of 210-230 beats per minute.

For 82 days, the animals were subjected to a disturbance of the daily rhythm of their surroundings [10]. At the end of this time they had become neurotic. In addition to various physiological disturbances, in three of them there was a change in heart rate: In two it had fallen to 115 beats per minute, and in the third, Khisar, to 75 per minute. Figure 2 shows the reduction in the heart rate in Barsuk, Khisar, and Abad (as measured by the EKG). Thus, before the neurosis, the heart rate was normal for apes of that age, but when neurosis developed, there was a marked bradycardia. Subsequently, when the apes were returned to a normal daily rhythm, and the disturbing circumstances were removed, the heart rate gradually returned to normal (see Fig. 2). The EKG of Khisar, in whom the bradycardia was very marked, is shown in Fig. 3.

The EKG shows many abnormalities characteristic of vagal tone. Thus, together with the bradycardia, a respiratory arrhythmia develops. In Fig. 3 (7) it can be seen that the R–R intervals vary. Normally, as has already been pointed out, this is not the case in apes. In Fig. 3 (1 and 2), the EKG of the same ape is shown before neurosis was induced, and it can be seen that the R–R intervals are all the same. In addition, the amplitude of all the waves is reduced; the T wave is flattened, and the Q–T interval greatly increased, as is particularly marked in Fig. 3, (2 and 7), where the leads from the chest show the T wave particularly well.

Precisely the same EKG changes have been described by A. F. Tur [8], G. F. Lang [4], and others for the so-called "vagus neurosis" in man ("vagal heart"). Cardiac conditions involving a slow pulse (bradycardia) have been described previously by V. F. Zelenin [2] and L. L. Fogel'son [9].

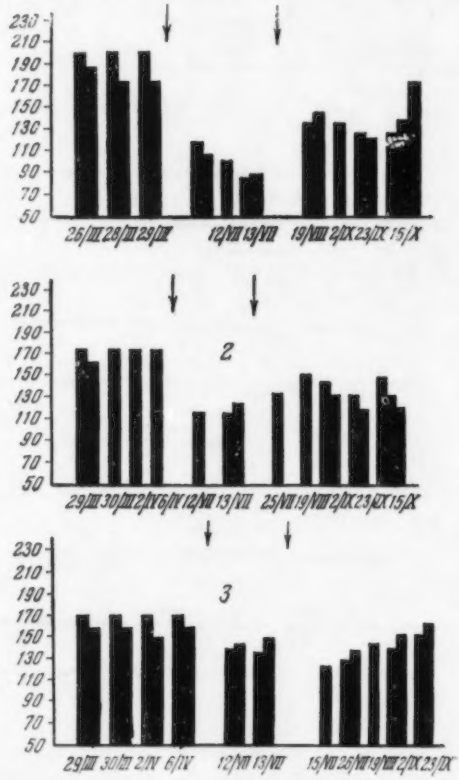

Fig. 2. Development of bradycardia during neurosis, and return to normal after the experiment. Above – in the ape Khisar; 2) in the ape Barsuk; 3) in the ape Abad. Heart rate is plotted upward; the arrows indicate the onset of the neurosis and the end of the experiment.

The method whereby the bradycardia develops may be represented as follows: Disturbance of the daily rhythm in apes induces a neurosis which is associated with severe disturbances in the functions of the internal organs [11]. The normal relationships between the processes of excitation and inhibition in the cerebral cortex are then upset, with the result that there is also a disturbance of the mutual inductive effects between cortex and subcortex. Normally, vagal tone is under cortical control [7]. Consequently, any change in cortical activity must react on the heart. The cortex exerts an inhibitory effect on the vagal center. Therefore, removal of cortical control leads to an increase in vagal tone. A. K. Smirnov has observed this phenomenon when removing the cerebral cortex in dogs [7]. K'ang K'ong-yeh [3] has shown that, when the cerebral cortex of pigeons is removed and vagal tone increased, there is a marked bradycardia and sinus arrhythmia. The reduction of the cortical control over the bulbar centers of the vagus nerve may also develop without operative interference, and may be caused by a neurosis which disturbs the normal cortical activity and its relationship with the subcortex. Consequently, vagal tone may be raised, as is shown by a marked bradycardia and sinus arrhythmia, and probably also by many disturbances of other systems.

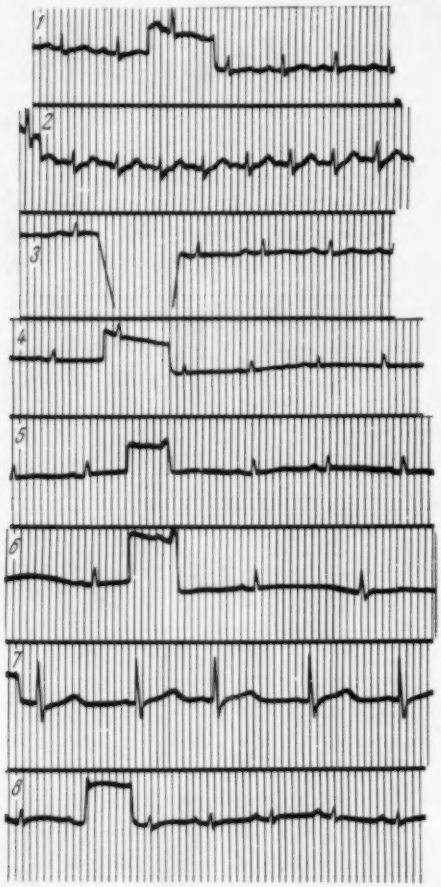

Fig. 3. Development of bradycardia and return of pulse to normal, as measured by EKG, in the ape Khisar. 1, 2) Initial EKG (lead II and CR_4; pulse – 200-184 beats per minute); 3) beginning of bradycardia (pulse 150 beats per minute); 4, 5, 6, 7) subsequent development of bradycardia (pulse – 120-100-86-75 beats per minute); 8) beginning of recovery (pulse – 150 beats per minute). Time-marker – 0.05 second.

SUMMARY

Studies have been made of changes in the heart rate in 79 apes, and it was found to decrease with age. The data were statistically significant. In adult apes a heart rate below 120 per minute indicates bradycardia, and above 240, tachycardia.

Neurosis was induced in 4 hamadryad baboons, aged two years, by disturbing their 24-hour rhythm; three developed bradycardia, and in one of them, the heart rate fell to 75 beats per minute, and a respiratory arrhythmia developed. The EKG changes were similar to those occurring in "vagal neurosis" in man. After the experiment, the heart rate returned to normal.

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DISTURBANCES OF THE VENOUS CIRCULATION OF THE PANCREAS IN THE PATHOGENESIS OF ACUTE HEMORRHAGIC PANCREATITIS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditiny,
Vol. 50, No. 8, pp. 50-54, August, 1960
Original article submitted July 25, 1959

There are reports in the literature that, in acute hemorrhagic pancreatitis, thrombosis of the large veins of the pancreas lying outside the organ, and especially of their small ramifications within the organ, is often observed [8, 15, and others]. Thrombus formation in the veins under these circumstances is connected with the action on the vascular wall of trypsin, liberated by death of the glandular tissue, and subsequent clotting of the blood [13, 17]. In addition to this secondary thrombosis, in accordance with Ricker's views [14], a primary thrombosis of the veins may also be postulated, as the result of neurovascular disturbances. The significance of primary venous thrombosis in the development of acute pancreatic necrosis is confirmed by individual observations from post-mortem examinations, and by the results of experimental work [4, 5, and others].

The existence of divergent views on this subject, and the inadequacy of its study, provided a motive for the present investigation. Since it is impossible to reproduce acute pancreatitis by an experimental model, with all its various causative factors and complicated reflex neurovascular mechanisms, we made it our aim to ascertain the importance of disturbances of the venous circulation of the pancreas in the development of acute hemorrhagic pancreatitis.

EXPERIMENTAL METHOD

The experiments, which were carried out on cats, consisted of ligation of the splenic vein, which was followed by thrombosis proximally to the site of the ligature. Thrombosis of the vein was produced by injection of substances into it which caused coagulation of the blood proteins (alcohol, tannin, acetic acid, vikasol – a vitamin K analog) or agglutination of erythrocytes. The latter was brought about by infusion of heterogenic blood (from a rabbit or white mouse) into the vein. In individual cases only the splenic vein was ligated. In all the experiments the spleen was removed as a preliminary measure. The operation was performed under ether anesthesia. Some animals died in the first few hours or days after the operation; others were sacrificed at different times: during the first few hours or after many days (see table).

Mortality among Animals after Ligation and Thrombosis of the Splenic Vein

Character of experiment	Number of animals		
	total in experiment	sacrificed	dying
Ligation of vein	4	4	–
Ligation of vein and thrombosis by means of:			
alcohol	23	17	6
tannin	43	19	24
acetic acid	6	4	2
vikasol	2	2	–
heterogenic blood	12	7	5
Total number of experiments	90	—	—

EXPERIMENTAL RESULTS

In those cases in which only the splenic vein was ligated, both at operation and at post-mortem (the animals were sacrificed after 1-3 days) the pancreas macroscopically appeared unchanged. Microscopically only a slight or moderate edema was observed, mainly in the interlobular stroma. When ligation of the splenic vein was followed by thrombosis, changes in the color of the organ were observed very soon after injection of the thrombosing substances, in the majority of cases at the operation itself. In particular, the "splenic part" of the pancreas to a considerable extent acquired a pinkish-red color with a slightly bluish tinge.

In the subsequent hours (especially during the first day) the pancreas, over a large extent, appeared greatly increased in volume, swollen, very firm, and dark red in color. The adjacent fatty cellular tissue was also swollen, with greatly congested vessels and focal hemorrhages (Fig. 1). The lumen of the splenic vein throughout its whole extent was filled with red thrombus. A blood-stained effusion was found in the peritoneal cavity. In individual cases, on the surface of the gland and in the surrounding fatty cellular tissue could be seen a few dull, whitish patches of fat necrosis. All these changes, but to a greater degree, were observed on the following days (on the 2nd-5th day after the experiment). Destruction and sequestration of the dead tissue in the gland were

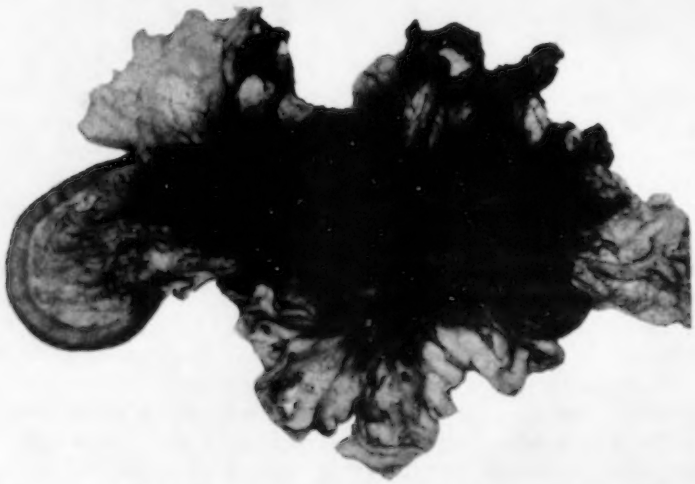

Fig. 1. General appearance of the pancreas in the first 24 hours after the experiment (ligation of the splenic vein and injection of 0.2 ml of tannin solution into its lumen). Side by side with the unchanged area of the gland, its "splenic part" is swollen, congested with blood, and dark red in color.

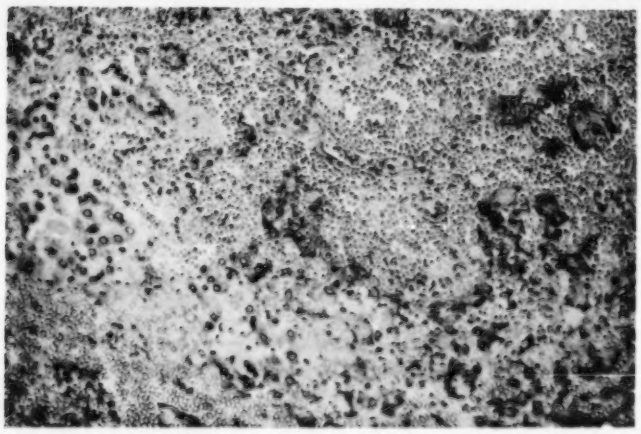

Fig. 2. Hemorrhages in the intralobular stroma. Disorganization and necrobiosis of the glandular cells. First 24 hours of the experiment. Moderate magnification. Stained by Van Gieson's method.

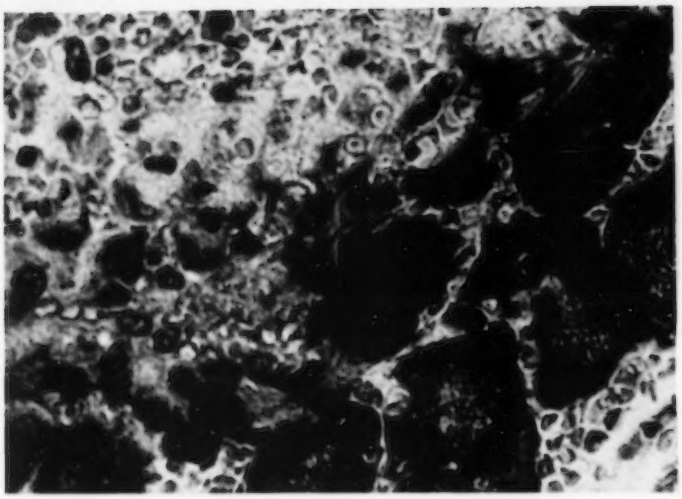

Fig. 3. Death of glandular tissue by means of gradual dissolution of the acinose cells (lysis of the nuclei and protoplasm of the cells). High power. Stained by Van Gieson's method.

later observed. At later periods (from 10 to 30 days) adhesions between the organ and the surrounding tissues were found in the region of the "splenic part."

The microscopic changes, like the macroscopic, were dependent on the degree of spread of the thrombosis into the branches of the splenic vein within the organ, and on the duration of the experiment. If the thrombosis was confined purely to the main trunk of the splenic vein, then, as in the case when the vein was ligated without injection of thrombosing substances, the changes consisted only of edema, mainly affecting the interlobular stroma. With spread of the thrombosis into the veins within the organ, considerable changes were observed in the stroma and parenchyma in the form of edema and hemorrhages in the interlobular and intralobular stroma, and of subsequent tissue and fat necrosis (Fig. 2). Studies of the trend of the changes at different periods of the experiment showed that the necrosis of the glandular tissue was secondary, i.e., it arose against the background of progressive hemorrhages by means of diapedesis. The gradual death of the glandular cells took place as a result of the lysis of their nuclei and protoplasm (Fig. 3). By the second or third day, considerable accumulations of leukocytes were observed in the foci of necrosis, and at later stages (6th-10th day), a fibroblastic reaction and gradual organization of the foci of necrosis were observed.

At about the end of the third week, an obvious fibrosis of the organ developed.

In discussion of the experimental results it must be remembered that the question of the localization of the primary changes in acute pancreatitis has found different answers in the literature. Some authors [6, 7, 12] considered that necrosis of the glandular tissue arises first, and then, in succession, changes develop in the stroma and vascular system in the form of edema, congestion, and hemorrhages. Experimental research in recent years [10, 11, 16], however, has clearly shown the primary character and the important role of circulatory disturbances in the development of changes in the parenchyma of the organ. The results of our experiments are in agreement with those obtained by Longo et al. [9], and show that glandular cell death set in secondarily, under the influence of "compressing" edema, hemorrhages, and anoxia. In our view, in acutely developing stasis of the blood, and subsequent hemorrhages, both the acinose cells and the capillary walls suffer damage. As a result of the disturbance of the "blood-tissue" barrier and of anoxia, a severe disturbance of the metabolic processes in the glandular cells takes place, which ultimately leads to progressive autodigestion of the organ. It must be emphasized that conditions such as these, which can lead to death of the glandular tissue and its autodigestion, are present only after spread of the thrombosis to the small branches of the splenic vein within the organ, whereas ligation or thrombosis of the main trunk alone does not lead to significant changes, on account of the numerous anastomoses in the portal vein system [1, 2, 3].

This pathogenesis of acute hemorrhagic pancreatitis which we have described is not common to all cases of acute involvement of the pancreas. Besides the hemorrhagic form of acute pancreatitis, for instance, are met edematous, necrotic, and suppurative forms, as well as pancreatic necrosis, with predominance of fat necrosis. In the development of these forms, other pathogenetic factors are of importance.

SUMMARY

Experimental induction of acute hemorrhagic pancreatitis in cats with the aid of venous thrombosis has demonstrated the importance of the pathogenetic role of acute venous circulatory disturbances within the organ in the development of this disease.

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REACTIVITY OF THE CARDIOVASCULAR SYSTEM IN EXPERIMENTAL NONINFECTIOUS PERITONITIS

Communication II. Reaction to the Intravenous Injection of Acetylcholine and Potassium Chloride

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 54-57, August, 1960
Original article submitted September 17, 1959

As we showed in the previous communication [1], in experimental noninfectious peritonitis, the character of the reaction of the cardiovascular system to the intravenous injection of adrenalin and caffeine is modified. In rabbits with peritonitis, a predominantly depressor reaction appears after the injection of these substances, i.e., the tone of the cholinergic part of the nervous system is observed to be increased.

In the present communication we have attempted to examine whether—and if so, how—the reaction of the cardiovascular system to the intravenous injection of acetylcholine and potassium chloride is modified in animals with peritonitis.

EXPERIMENTAL METHOD

The experiments, which were carried out on rabbits, consisted of four series, with 5 rabbits in each series. In the first series, we studied the reaction of the cardiovascular system of healthy animals to the injection of acetylcholine (0.002 mg/kg body weight) and in the second series, to the injection of potassium chloride (2 ml of a 2% solution). In the third and fourth series, 18-20 hours before the experiment, noninfectious peritonitis was induced in the rabbits by means of the method described in the previous communication [1].

EXPERIMENTAL RESULTS

During the analysis of the pattern of changes in the blood pressure, we took into account the duration of the phase of lowering the pressure, its minimum level, the duration of restoration of the original pressure, and the magnitude of the fall in pressure from the initial level.

These results are shown in Tables 1 and 2.

Analysis of the data shows that, in rabbits with peritonitis after injection of acetylcholine, the arterial pressure fell considerably and reached its minimum level more quickly than in the control animals; the fall in pressure in these animals was almost 2.5 times greater than in the controls. Another characteristic feature of the change in the blood pressure in rabbits with peritonitis was the sharp retardation (five-fold) of its return to its initial level (Fig. 1).

In response to the injection of acetylcholine, the cardiovascular system in rabbits with peritonitis thus reacted by a greater fall in the arterial pressure and also by a slower return to its original level.

It can be seen from an analysis of the changes in the arterial pressure after injection of potassium chloride that, in rabbits with peritonitis, it reached its minimum level slightly more slowly. However, this level was lower than in the control animals. The degree of the fall in pressure in the rabbits with peritonitis was considerably greater than in the controls, but the time of its return to the initial level was only two-thirds of that in the controls (Fig. 2).

Thus, in rabbits with peritonitis, in response to the intravenous injection of potassium chloride, the blood pressure fell much more than in healthy rabbits, but the fall developed slightly more slowly.

The results described show that, in rabbits with peritonitis, after injection of potassium chloride, the

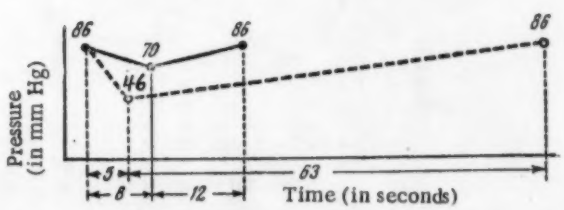


Fig. 1. Trend of the changes in the arterial pressure in healthy rabbits (——) and rabbits with peritonitis (- - -) after intravenous injection of acetylcholine.

TABLE 1. Values Showing the Pattern of the Changes in Blood Pressure of Rabbits after Injection of Acetylcholine

Limits of variation (A) and mean value (B)	Initial level (in mm Hg)	Time taken to reach minimum (in sec)	Minimum level (in mm Hg)	Time taken to return to initial level (in sec)	Magnitude of fall (in mm Hg)
Control group					
A	80-90	2-11	60-80	9-16	10-20
B	86	8	70	12	16
Experimental group (with peritonitis)					
A	75-90	4-6	42-50	56-72	30-50
B	86	5	46	63	40

TABLE 2. Values Showing the Pattern of the Changes in Blood Pressure of Rabbits after Injection of Potassium Chloride (height in mm Hg, time in seconds)

Limits of variation (A) and mean value (B)	Initial level	Time taken to reach minimum	Minimum level	Time to return to initial level	Magnitude of fall
Control group					
A	80-120	2-5	60-80	8-49	25-40
B	96	3	65	27	31
Experimental group (with peritonitis)					
A	70-120	2-9	40-60	15-30	30-60
B	92	5	53	18	39

arterial pressure fell by 26%, and after injection of acetylcholine, by 150% more than in the controls (Fig. 3).

In rabbits with peritonitis, the cardiovascular system thus responds to the injection of these drugs by a more intensive depressor reaction than occurs in healthy animals.

According to the modern view, the action of acetylcholine on the cardiovascular system is based on the direct stimulation of chemoreceptors by the drug [3, 4, 7], i.e., the action is reflex in nature. The character of its effect may differ, however, depending on the state of the central nervous system. D. N. Lazareva [2] for instance, found that after the intravenous injection of carbaminoylcholine chloride into rabbits infected with typhoid fever, a much greater slowing of the heart rate and a much quicker fall in the arterial pressure take place than in control animals. L. G. Reznichenko [5] states that after mesenteric trauma, or hemorrhage, in cats the intravenous injection of acetylcholine raises the arterial pressure, whereas in control animals the pressure is lowered.

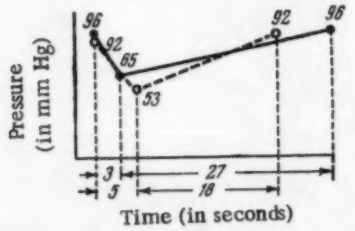
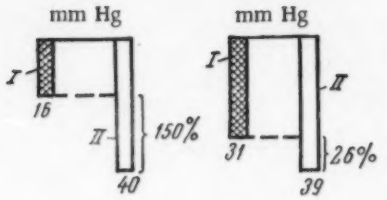


Fig. 2. Trend of the changes in the arterial pressure in rabbits after intravenous injection of potassium chloride. Legend as in Fig. 1.

Our results also showed that, in rabbits with peritonitis, the reaction of the cardiovascular system after the intravenous injection of acetylcholine and potassium chloride differs from its reaction in healthy rabbits. This difference is characterized by the fact that after injection of acetylcholine the blood pressure falls more rapidly and to lower figures, but then returns more slowly to its initial level. After injection of potassium chloride, the pressure falls more slowly and intensively, but returns more rapidly to its original level.

As G. M. Shpolyanskii [8] points out, in peritonitis, and especially in the second phase, a paretic state of the sympathetic nervous system develops. This has also been shown experimentally by S. D. Sokolov [6], who found that an isolated segment of bowel in a dog with



a b

Fig. 3. Magnitude of the fall in the arterial pressure after injection of acetylcholine (a) and potassium chloride (b) in control (I) and experimental (II) rabbits.

peritonitis reacts much more strongly to choline and physostigmine than in a healthy animal, whereas the reaction to adrenalin is significantly weakened. This author considers that the sympathetic innervation of the inflamed intestine loses its excitation sooner than the parasympathetic supply, and that the latter exerts a stronger influence than the sympathetic. D. N. Lazareva [2] found an increase in the tone of the cholinergic nervous system, and also a lowering of the cholinesterase activity in rabbits infected with typhoid fever.

It may also be considered from our experimental results that, in rabbits with peritonitis, the tone of the cholinergic nerves is increased; the increase in the duration of the return of the blood pressure from its minimum to its initial level after the injection of acetylcholine may be accounted for by depression of the cholinesterase activity. However, this problem requires further study.

SUMMARY

The author studied the reaction of the cardiovascular system in rabbits in response to the intravenous injection of acetylcholine and potassium chloride in experimental noninfectious peritonitis. It was shown that the depressor effect on the cardiovascular system induced by these substances was more pronounced against the background of peritonitis, which possibly points to the increased tone of the cholinergic nerves.

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*See English translation.

MECHANISMS OF DISTURBANCES OF RESPIRATION AND THE CIRCULATION DURING HIGH SPINAL ANESTHESIA*

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Translated from Byulleten'Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 57-62, August, 1960
Original article submitted August 30, 1959

Among the various types of anesthesia, spinal anesthesia occupies an important place. The physiological mechanisms of the respiratory and circulatory disturbances which are the principal danger of high spinal anesthesia have not been adequately studied; views regarding their pathogenesis are contradictory, and no agreement has been reached on the question of methods of their prevention and management.

The authors of the majority of modern textbooks and monographs claim that respiratory arrest and a fall in the blood pressure are the results of spread of the anesthetic solution into the fourth ventricle and of paralysis of the bulbar centers [2, 16, 21, 28, 31]. Other researchers [1, 9, 10, 22] strongly consider that the anesthetic has a paralyzing action on the peripheral motor and vasomotor conductors. V. A. Petrov [10], Babcock [22], MacIntosh [29], and others attach the main importance to the respiratory disturbances. A. F. Popova [12], S. A. Rusanov [15], and Thorek [32] consider that the fall in blood pressure is primary, and regard the respiratory arrest as the result of inadequacy of the circulation in the centers of the medulla.

In B. E. Frankenberg's opinion [20], a group of connected and mutually aggravating factors is concerned in the pathogenesis of the respiratory and circulatory disturbances during high spinal anesthesia. In anesthesia at levels up to the first thoracic segment, all the roots of the intercostal nerves are blocked, and the vasoconstrictor sympathetic innervation is interrupted. The intercostal muscles are excluded from the act of breathing, which is maintained by the diaphragm alone. The fall in the blood pressure resulting from the preganglionic block of the vasoconstrictors is aggravated by the decrease in the pumping power of the chest, by the reduction in the venous return flow to the heart, and by the fall in the systolic volume. During spread of the anesthetic to the region of the superior cervical segments, the roots of the phrenic nerves are blocked, and total respiratory arrest develops. The anoxemia which ensues under these circumstances leads to anoxia of the bulbar centers, with a subsequent secondary, catastrophic fall of the blood pressure.

Clinical observations on the course of high spinal anesthesia by B. E. Frankenberg's method [20] show that preliminary subcutaneous injection of caffeine with ephedrine, and subarachnoid injection of ephedrine, prevent or considerably minimize the primary fall in the blood pressure associated with blocking of the vasoconstrictors, but do not prevent the disturbances of respiration. In 2000 cases of spinal anesthesia performed during the last ten years, we observed respiratory arrest in 18 patients in conjunction with a stable, or even increased (above the initial level), blood pressure. The period of apnea lasted from 15 to 45 minutes. In all cases artificial respiration was applied by means of the breathing bag of the anesthetic machine. In 16 patients the blood pressure remained stable throughout the period of apnea. In two patients a fall in blood pressure was observed on account of the delayed application of artificial respiration; when this was applied, the blood pressure was restored.

The possibility of the development of apnea without a fall in the blood pressure suggests that circulatory failure in the medullary centers cannot be regarded as the principal cause of the respiratory disturbances during high spinal anesthesia. The ensuring of pulmonary ventilation by means of artificial respiration prevents a secondary fall in the blood pressure and ultimately has a decisive influence on the outcome of the spinal anesthesia.

These clinical observations led us to make an experimental study of the pathogenesis of the respiratory and circulatory disturbances during high spinal anesthesia, and of the efficacy of artificial respiration as a method of dealing with these disturbances.

EXPERIMENTAL METHOD

Experiments were carried out on dogs. The results of 95 experiments, grouped in five series, are considered.

* Address given at the conference on clinical physiology convened by the AMN SSSR in Moscow on May 20, 1959.

For the subarachnoid injection of the anesthetic, we used the two following methods: 1) the dura mater was exposed in the lumbar region by means of laminectomy, and punctured under direct vision; 2) the atlantooccipital membrane was exposed and dissected, and through an opening made in the membrane, a No. 5 ureteric catheter was passed in the caudal direction as far as the lumbosacral division, through which the solution was injected. Post-mortem examination of the skull and spine in experiments in which a colored solution was injected (methylene blue), and roentgenological examination of the spread of a contrast solution (sergosine) showed that, by the first method, the anesthetic floods the subarachnoid space of the spinal cord and fills the ventricular system; in the second case, the anesthetic escapes through the opening in the membrane and does not reach the fourth ventricle. The blood pressure was recorded from the carotid artery and respiration from the trachea (through a tracheotomy tube); the laminectomy, introduction of the catheter, tracheotomy, and manipulation of the carotid artery were carried out under morphine-ether anesthesia. The spinal anesthetic was injected after the interval which was required to bring the animal round from the general anesthesia.

EXPERIMENTAL RESULTS

In the first series of experiments (20 dogs) 4 ml of 2% procaine was injected into the spinal subarachnoid space. Irrespective of the method of injection, considerable respiratory disturbances developed, followed by a catastrophic fall of the blood pressure. Respiratory arrest in all cases preceded the cessation of the heart beat. Attempts to restore the breathing by the suboccipital injection of procaine, which, according to D. Ya. Daron [6], Flagg [24], and others, is an effective method of stimulation of the respiratory center, were unsuccessful in our experiments. Nineteen of the 20 dogs (95%) died.

In the second series of experiments (19 dogs) we studied the reaction of the respiration and circulation to direct contact of the anesthetic with the medulla. The solution was injected by suboccipital puncture. The dog lay in the prone position, with its head hanging over the operating table, forming almost a right angle, open forward, in the cervical region of the spine. Post-mortem examination showed that, when injected in this manner, the colored solution filled the system of the ventricles and did not descend into the subarachnoid space of the spinal cord.

Doses of procaine which were lethal when injected into the subarachnoid space of the spinal cord (2 ml of 4% procaine) did not lead to arrest of respiration. In contrast to the view most widely held in the literature, that the anesthetics have a paralyzing effect on the vasomotor center, a considerable rise of blood pressure was observed (Fig. 1). The subsequent suboccipital injection of caffeine brought about stimulation of respiration and a new pressor reaction. In our opinion, this result showed that the anesthetic does not block the bulbar centers and does not prevent them from reacting to the injection of stimulants. It must be emphasized that we deliberately used concentrations of procaine which were far higher than any which might reach the fourth ventricle in clinical conditions (about 0.1% according to Green [26], 0.021% according to Koster [27]).

In view of the possibility of diffusion of the anesthetic into the subarachnoid space of the skull [29], the rise of blood pressure after the suboccipital injection of the solution might be regarded as the consequence of a "chemical vagotomy," abolishing the inhibitory action of the vagus nerves on the cardiovascular system. The suboccipital injection of procaine in four experiments in association with bilateral vagotomy, however, led to a no less marked pressor reaction. We suggest that the rise in blood pressure in response to the suboccipital injection of procaine depends on the direct stimulation of the vasomotor center by the anesthetic. This agrees to some extent with the viewpoint of M. A. Bubnov [3], who produced pressor reactions by injection of procaine into the carotid artery.

The results of the first two series of experiments thus suggest that respiratory arrest and the fall in blood pressure during high spinal anesthesia are the result of the action of the anesthetic on the peripheral conductors and not on the bulbar centers.

In the third series of experiments (17 dogs) we injected 4-5 ml of 2% procaine into the subarachnoid space of the spinal cord (a dose which was lethal to 95% of the dogs of the first series), and on the appearance of respiratory disturbances, the animal was changed over to artificial respiration by means of the usual apparatus used in the physiological laboratory. Sixteen of the 17 dogs in this series survived. The application of artificial respiration resulted in stabilization of the blood pressure and prevented death of the experimental animal.

In the fourth series of experiments (20 dogs) the same usually lethal dose was injected along the catheter into the subarachnoid space. On the first signs of impending respiratory arrest, electrophrenic respiration was applied [17, 23, 30]. In 17 experiments we used an apparatus for electrical stimulation of the phrenic nerves constructed in the workshops of the Leningrad State Postgraduate Medical Institute (GIDUV), and, in three experiments, an improved apparatus supplied to us for testing by the Moscow experimental factory of the All-Union Research Institute of Medical Instruments and Apparatus of the Ministry of Health of the USSR [19]. Rhythmic stimulation of the motor points of the phrenic nerve in the skin of the neck in all cases led to rhythmic contraction of the diaphragm—contraction of a given depth and frequency. We used rhythmic interrupted impulses of current with a frequency of 50 to 80

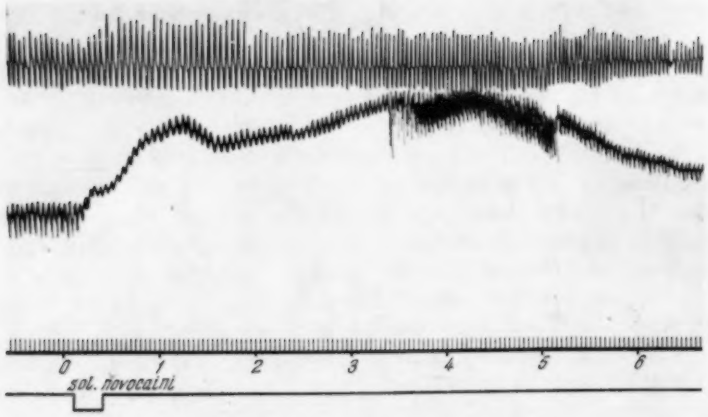

Fig. 1. Recording of the respiration and blood pressure after the suboccipital injection of 2 ml of a 4% procaine solution. Time marker – 3 seconds.

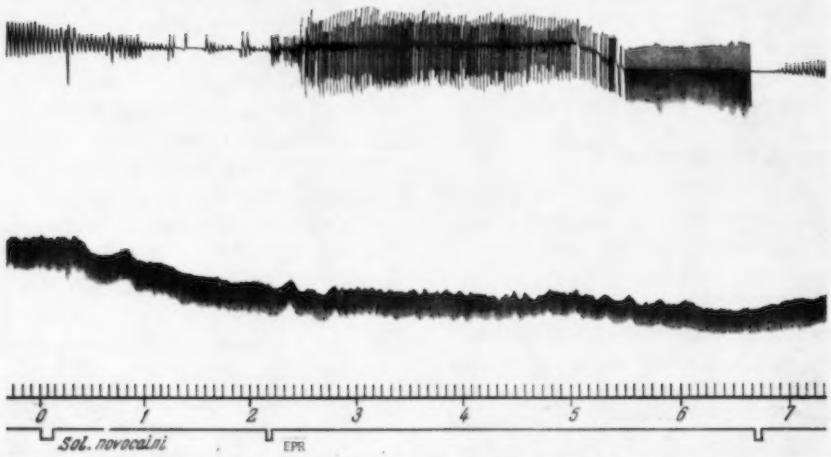

Fig. 2. Recording of respiration and blood pressure after subarachnoid injection of 4 ml of a 2% procaine solution into the lumbar division, followed by the use of electrophrenic respiration (at the third minute). At the eighth minute spontaneous respiration was restored. Time marker – 5 seconds.

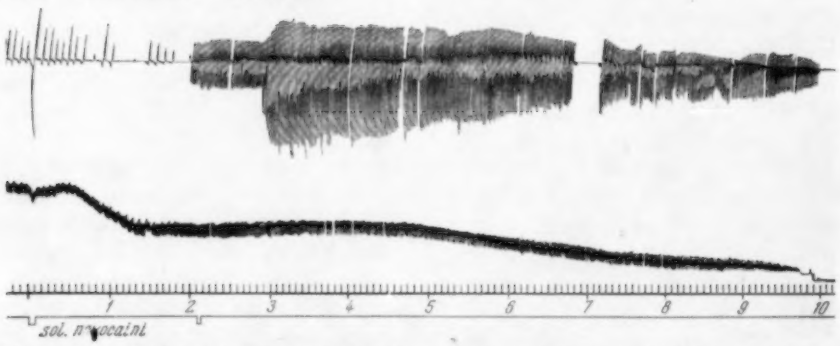

Fig. 3. Recording of the respiration and blood pressure after the subarachnoid injection of 4 ml of a 2% procaine solution into the lumbar division after bilateral vagotomy. At the third minute the animal was changed over to electrophrenic respiration, but spontaneous breathing was not restored. Time marker – 5 seconds. The second stimulation marker corresponds to the application of EPR.

cps and a voltage of 2.5 to 7.0 v at the poles of the electrodes.

Electrophrenic respiration in all the experiments led to stabilization of the blood pressure and to the restoration of spontaneous breathing (Fig. 2). All the dogs of this series survived.

In order to define more precisely the mechanisms involved in the restoration of the vital functions under the influence of artificial respiration, at Prof. R. O. Faitel'berg's suggestion, we carried out a series of experiments (19 dogs) in which the vagus nerves were divided. These nerves are known not to be blocked by even the highest spinal anesthesia [7, 8, 13]. After unilateral vagotomy, artificial respiration led to the restoration of spontaneous breathing in nine of the ten experimental animals. With bilateral vagotomy, artificial respiration was ineffective (Fig. 3). All nine dogs died.

Afferent impulses from the receptors of the respiratory system play a considerable part in the regulation of respiration [4, 11, 14, 18]. These impulses are conducted along the vagus nerves. The combination of high spinal anesthesia with bilateral vagotomy essentially amounts to complete isolation of the respiratory center from afferent impulses, which leads to the development of a state of atony of the center. Artificial respiration after bilateral vagotomy does not restore the flow of afferent impulses from the receptors of the respiratory system and does not overcome the atonic state of the center. The mechanically induced ventilation and gas exchange is insufficient for restoration of the vital functions. We attribute the death of the vagotomized animals to the exclusion of the reflex stimulating role of the artificial respiration.

SUMMARY

Respiratory and circulatory disturbances during high spinal anesthesia were made the subject of an experimental study. The dominating role of respiratory disturbances results from the action of the anesthetic upon the peripheral conductors. Direct contact of the anesthetic with the medulla oblongata not only fails to induce bulbar paralysis, but acts as an excitant on the vasomotor center. The maintenance of respiratory functions by artificial respiration is of decisive significance for the outcome of the spinal anesthesia.

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THE EFFECT OF ADRENALIN ON HISTAMINE METABOLISM IN NORMAL CONDITIONS AND IN DISEASES OF THE DIENCEPHALON

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 62-67, August, 1960

Original article submitted October 13, 1959

In our research [4] on the study of histamine metabolism in certain forms of disease of the diencephalon, we found that absence of a histaminopectic effect, i.e., the ability of the serum to inactivate histamine added to it in vitro, is a characteristic feature of patients with lesions of the diencephalic region. The variations in the content of histamine and diamine oxidase in these diseases do not exceed normal limits. Meanwhile, in experiments on animals [1] to study the relationship between the metabolism of histamine and adrenalin under the influence of factors causing stress, it was shown that the content of histamine and adrenalin-like substances (ALS) in the blood, the brain, and lung tissues, and the mucous membrane of the intestine, varies in different ways in these cases. Whereas the histamine content of the brain and lung tissues was reduced in response to an increase in the ALS level, the contents of ALS and histamine in the blood and the intestinal mucosa were simultaneously increased.

According to data in the literature, stimulation of the sympathetic nerve [9] or the intravenous injection of adrenalin into dogs [8] causes a transient increase in the histamine content of the blood. Similar changes are also observed in human patients suffering from various diseases (bronchial asthma, gastric ulcer, bronchitis). It has been found that, in response to the intravenous injection of adrenalin into the patients, the histamine content of the blood is increased, returning to its initial level after 10 minutes [10].

In the development of the work undertaken by our group on the study of the relationship between the clinical manifestations and the associated neurohumoral changes [2, 5, 6, 7] in the present communication, we report the results of an investigation of the histamine metabolism in the blood of healthy persons and patients under the influence of exogenous adrenalin. We used the latter as one of the functional tests to judge the limits of homeostatic reactions in the healthy and sick human subject.

EXPERIMENTAL METHOD AND RESULTS

The subjects under observation received subcutaneous injections of adrenalin (0.3- 0.5 ml of a 0.1% solution). Blood for examination was taken from the cubital vein before the injection, and 10 or 20 minutes thereafter. Estimations were made of the histamine, the diamine oxidase, and the histaminopectic effect of the blood by means of methods previously described [4].

Observations were made on 20 patients with lesions of the diencephalic region. As a control group we used healthy subjects (33 persons) and patients with various other forms of nervous disease (11 persons).

In accordance with N. I. Grashchenkov's classification [3], nine patients with a diencephalic syndrome belonged to a group in which vegetative vascular manifestations were predominant; in 11 patients there was a well-marked neuroendocrine syndrome.

The results obtained in the healthy subjects are shown in Table 1.

In eight of the ten healthy subjects who received an injection of 0.3 ml of adrenalin, the histamine content of the blood 10 minutes after injection was lower than that before the injection. In only one case was an increase in the histamine content observed, and in this case, moreover, a considerable increase in the diamine oxidase activity was present. In all the remaining subjects the diamine oxidase activity was either very slightly increased or remained unchanged. The histaminopectic effect was unchanged under the influence of the injection of adrenalin. If the blood for examination was taken 20 minutes after the injection of adrenalin, the changes in the histamine content were less pronounced; in four of the eight healthy subjects so tested, the histamine content was not decreased, but increased. If the volume of adrenalin injected was increased to 0.5 ml, the histamine content in the blood of the subjects in this group also fell 10 minutes after injection, but after 20 minutes there developed a slight tendency for this content to increase.

TABLE 1. Histamine Content, Diamine Oxidase Activity, and Histaminopectic Effect in the Blood of Healthy Human Subjects

Dose and time of second blood sampling	Patient No.	Histamine (in μg%)		Diamine oxidase (in μg/ml)		Histaminopectic effect (in %)	
		before adrenalin injection	after adrenalin injection	before adrenalin injection	after adrenalin injection	before adrenalin injection	after adrenalin injection
0.3 ml 10 min	1	5.6	3.0	0	0	17	17
	2	17.2	3.6	2.0	2.0	28	28
	3	5.6	4.6	0	0	—	33
	4	8.2	5.0	0	0	25	25
	5	5.2	10.6	0	13	55	55
	6	4.0	4.0	3.0	3.5	47	52
	7	16.0	6.0	1.2	1.5	—	—
	8	13.0	11.0	0	0	30	30
	9	4.0	3.4	2.0	3.0	50	47
	10	12.4	10.4	4.0	8.0	38	—
0.3 ml 20 min	11	8.6	11.7	1.0	1.0	25	25
	12	11.6	10.2	0	0	31	22
	13	7.2	9.6	0	0	24	30
	14	11.6	10.2	2.0	2.0	30	30
	15	5.6	3.0	0	0	25	25
	16	16.0	20.0	2.0	0	25	25
	17	3.0	3.6	0	4.4	40	40
	18	6.6	5.0	3.6	0	49	48
0.5 ml 10 min	19	6.8	4.6	0	1.2	0	1
	20	4.6	3.4	5.4	6.0	41	38
	21	8.0	4.6	2.8	2.2	43	40
	22	7.6	3.8	0	0	20	24
	23	6.8	4.2	7.0	3.2	45	40
	24	10.4	15.0	0	0	30	28
	25	17.6	10.4	0	0	36	36
0.5 ml 20 min	26	15.6	16.0	2.0	2.0	30	28
	27	5.0	11.2	3.0	0.0	36	28
	28	4.6	5.2	—	—	36	20
	29	5.0	9.3	3.0	5.0	27	22
	30	7.2	7.6	0	0	10	14
	31	7.0	8.6	0	0	40	59
	32	6.6	7.6	0	3.0	41	35
	33	11.2	13.2	0	0	17	27

After the injection of histamine in these doses, in the majority of healthy subjects (30 persons) no obvious clinical reaction was observed, and the general condition remained satisfactory. A well-marked clinical reaction, manifesting itself in the form of headache, shivering, rigors, and changes in the arterial pressure, pulse, and respiration, was observed in only three persons who received adrenalin in a dose of 0.5 ml, which coincided with an increase in the blood-histamine concentration (Nos. 24, 27, and 29). In four subjects only an increase in the arterial pressure was observed. At later stages (after 30-40 minutes) a partial or clear-cut clinical reaction was observed in 19 of 30 subjects.

In Table 2 we give the results obtained from investigation of these indices in patients with different forms of nervous disease.

In contrast to the results obtained in the healthy subjects, in diencephalic diseases (in 14 of 15 cases) a clinical reaction was observed 10 minutes after the injection of 0.3 ml of adrenalin. In 11 patients the blood-histamine concentration was increased, and in four it was lowered. The diamine oxidase activity was unchanged or lowered, but the serum histaminopectic effect showed no significant change. In most subjects a perceptible increase in the blood-histamine level coincided with the onset of a clearly marked clinical reaction.

The histamine content was very slightly lowered in four of the five patients from whom blood for the second examination was taken 20 minutes after the injection of 0.3-0.5 ml of adrenalin. In seven of eleven patients with other forms of nervous disease, a small increase in the blood-histamine content was observed after the injection of adrenalin, in the absence of changes in the diamine oxidase and the histaminopectic effect. At the time of taking blood, a clinical reaction was observed in only two of these patients (Nos. 24 and 30).

As the results described show, the changes in the blood-histamine content under the influence of small doses of adrenalin, both in healthy subjects and in pa-

TABLE 2. Histamine Content, Diamine Oxidase Activity, and Histaminopectic Effect in the Blood of Patients with Various Forms of Nervous Disease

Dose and time of second blood sampling	Patient No.	Diagnosis	Histamine (in μg %)		Diamine oxidase (in μg/ml)		Histaminopectic effect (in %)	
			before injection of adrenalin	after injection of adrenalin	before injection of adrenalin	after injection of adrenalin	before injection of adrenalin	after injection of adrenalin
	1	Diencephalic syndrome	7.2	20.0	2.0	0	16	16
	2	The same	8.6	12.4	8.0	4.0	13	10
	3	" "	4.2	8.0	–	–	0	0
	4	" "	7.4	16.6	4.0	4.0	0	0
	5	" "	6.6	9.0	5.8	4.4	0	0
	6	" "	8.6	5.6	0	1.8	0	0
0.3 ml, 10 min	7	" "	6.0	7.1	1.0	1.2	0	0
	8	" "	5.6	4.0	0	0	0	0
	9	" "	5.0	3.5	0	1.4	20	20
	10	" "	6.0	7.0	0	0	0	0
	11	" "	7.6	4.0	9.0	2.0	0	0
	12	" "	15.0	18.0	0	0	8	10
	13	" "	10.2	17.6	0	0	0	0
	14	" "	9.2	13.4	4.6	4.6	0	0
	15	" "	7.4	11.2	0	0	0	0
	16	" "	6.0	4.0	0	1.0	0	0
0.3-0.5 ml, 20 min	17	" "	14.8	11.8	0	6.0	–	–
	18	" "	9.6	10.6	5.0	3.0	0	0
	19	" "	9.2	9.0	0	0	0	0
	20	" "	6.6	5.6	2.0	2.0	–	–
	21	Vegetative vascular dystonia and neurasthenia	3.2	4.6	1.4	–	0	0
	22		12.4	8.6	9.4	10.6	0	0
	23		10.2	9.8	1.2	1.2	0	0
	24	Residual manifestations of arachnoencephalitis	6.2	3.0	4.2	1.4	12	18
	25		16.6	24.4	1.6	1.8	0	0
	26		4.6	9.0	0	0	–	–
0.3 ml, 10 min	27	Narcolepsy	10.0	11.0	9.0	3.0	0	0
	28	Lipodystrophy	4.8	8.2	1.5	4.6	20	19
	29	Allergy	13.9	8.2	10.4	8.0	–	–
	30	Disease of the muscles	14.5	15.0	0	0	–	–
	31	Adenoma of the adrenals	7.5	8.6	1.0	1.0	0	0

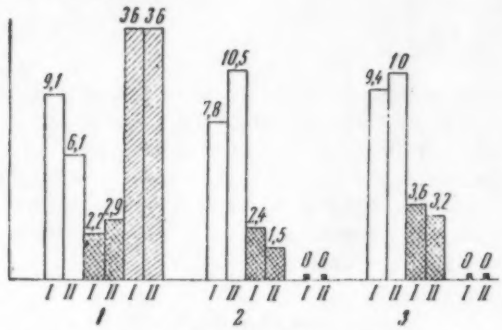
tients with lesions of the diencephalon, are consistent in character. Ten minutes after the injection of 0.3 ml of adrenalin, a fall in the blood-histamine content (from 9.1 to 6.1 μg % on the average) was observed in the majority of healthy subjects, with a small increase in the diamine oxidase activity (from 2.2 to 2.9 μg/ml) and in the absence of changes relating to the histaminopectic effect (see figure).

In contrast to these findings, in patients with diencephalic lesions, after injection of adrenalin, the blood-histamine content rose (from 7.8 to 10.5 μg % on the average) with a slight fall in the diamine oxidase activity (from 2.4 to 1.5 μg/ml). In patients with other forms of nervous disease (vegetative vascular dystonia, allergy, residual manifestations of arachnoencephalitis, diseases of the muscles, and so on), in the majority of cases, the same trend was observed in the histamine metabolism as in patients with the diencephalic syndrome; these changes, however, were far less pronounced.

A characteristic finding was that in 14 of the 15 patients with the diencephalic syndrome, an obvious

clinical reaction was observed 10 minutes after the injection of adrenalin.

It has been shown [4-8] that the liberation or formation of free histamine is often connected with the sympatho-adrenal regulatory system. The brief increase in the blood-histamine content, followed by its rapid fall to a level close to its initial value, may be regarded as the reaction of the subject to the injection of adrenalin. In patients with disease of the diencephalon, this reaction is evidently disturbed, and moreover, the process of restoration of the initial level of the blood histamine is also delayed.



Histamine content, diamine oxidase activity and histaminopectic effect in the blood of healthy subjects and patients, before the injection of 0.3 ml of a 0.1% solution of adrenalin, and 10 minutes thereafter. Unshaded columns – mean blood-histamine level (in μg %); cross-hatched columns – mean level of diamine oxidase activity (μg/ml); obliquely shaded columns – mean value of histaminopectic effect (in %); I) before injection; II) after injection; 1) normal; 2) disease of the diencephalon; 3) various forms of nervous disease.

SUMMARY

This work deals with the changes induced by adrenalin injections in the histamine content, diamine oxidase activity, and histaminopectic effect in healthy individuals and in patients with diencephalic afflictions. Adrenalin administration served as a functional test to assess the limits of homeostatic reactions in healthy and sick persons. In healthy individuals, the blood-histamine content decreases, and the activity of diamine oxidase increases slightly, 10 minutes after subcutaneous injection of 0.3 ml of 0.1% adrenalin solution; as to the histaminopectic effect – no changes are seen. As distinct from the above, in patients with a diencephalic syndrome the blood-histamine content rises and the activity of diamine oxidase decreases somewhat. These changes, accompanied by a clinical reaction, are characteristic of some forms of diencephalic pathology and may be used for diagnosis.

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THE EFFECT OF ADENINE NUCLEOTIDES ON THE PERMEABILITY OF THE SKIN VESSELS IN RATS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 67-70, August, 1960
Original article submitted June 27, 1959

Of great importance in the pathogenesis of inflammation are the so-called "endogenous factors" of inflammation, which are physiologically active products of metabolism and tissue destruction. They include histamine, acetylcholine, sympathin, serotonin, and a group of polypeptides (leukotaxin, bradykinin), active proteins, and products of nuclein metabolism.

An increase in the permeability of the vessels, along with the emigration of leukocytes, is one of the earliest signs of inflammation. Numerous observations [3, 5, 6, 7, 9, 10] have shown that during the action of an inflammatory agent—trypan blue, when injected intravenously, rapidly penetrates into the focus of inflammation. The role of several different factors (histamine, leukotoxin, hyaluronidase) in the increasing of the vascular permeability in inflammation has also been investigated.

We have found [2, 4] that, together with other physiologically active substances, the adenine nucleotides play a definite role in the pathogenesis of the principal phenomena of inflammation — in the disturbance of the vascular permeability, in the emigration of the leukocytes, and in their phagocytic function. In the early stage of development of inflammation, there is an increase in the ATP content of the blood flowing from the focus of inflammation, and also in the inflamed tissue itself.

In investigations on rabbits we demonstrated the importance of the adenine nucleotides in the disturbances of vascular permeability. We found that ATP causes a marked increase in the permeability of the skin vessels in normal animals. For instance, after the intradermal injection of 1000-200 γ of ATP (sometimes after 20 γ)—if, at the same time, a 1% solution of trypan blue (5 ml/kg body weight) is injected intravenously,—the dye is rapidly deposited in the area of the skin into which the ATP was injected. The intensity of deposition of the dye in these areas is largely determined by its concentration in the blood. With a decrease in the amount of dye injected (2.5-2.0 ml/kg body weight), the reaction develops later and is weaker. A dose of 1 ml/kg does not produce the concentration in the blood necessary for the rapid detection of its passage into the tissue, and is therefore unsuitable for this purpose.

In order to continue the study of the problem of the increase in the vascular permeability under the influence of compounds of the adenylic system, we conducted a series of observations on the permeability of the skin vessels in white rats.

EXPERIMENTAL METHOD

Experiments were carried out on 40 white rats weighing 140-170 g, in which the hair of the skin of the abdomen had been clipped 24 hours beforehand. An intraperitoneal injection of 0.8-1.0 ml of a 1% solution of trypan blue was given. After an interval of 3-4 minutes had elapsed, ATP or adenylic acid in a dose of 1000-250 γ (occasionally 25 γ ATP) in 0.1 m' of physiological saline was injected into the skin of the abdomen (at a distance of 1 cm from the linea alba). The solutions were prepared at the moment of injection. ATP preparations from the Ivanovo Meat Combine, and also imported adenylic acid and MAP in ampules, were used. In control experiments, 0.1 ml of physiological saline was injected. In order to achieve greater clarity, in some experiments the solutions of ATP or adenylic acid were injected into the skin on one side of the abdomen, and physiological saline was injected into the symmetrically opposite skin. Increased permeability of the vessels was judged by the intensity of staining of the area of skin into which the test substances were injected. Observations were maintained for a period of 2 hours.

EXPERIMENTAL RESULTS

From 10 to 15 minutes after the injection of ATP (in some cases after 30 minutes) the dye appeared in the skin around the site of injection. The intensity of staining gradually increased, and the dye spread diffusely over the whole area, to reach maximum saturation after 1.5-2 hours (Fig. 1). The diameters of the stained areas in the course of a marked reaction to injection of 250, 500, and 1000 γ ATP were 11-14 mm. In response to

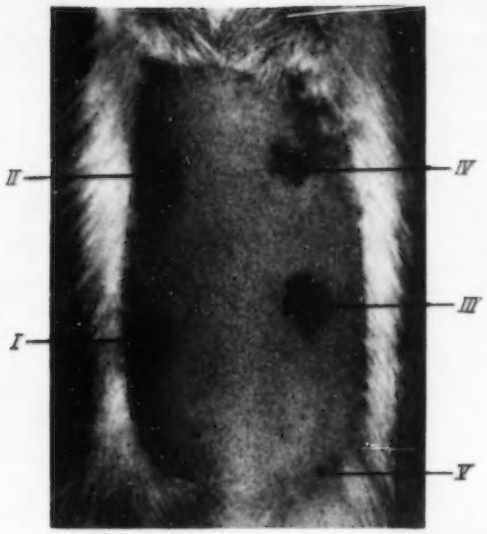

Fig. 1. Deposition of trypan blue, after injection of 1 ml of a 1% solution in areas of the skin of the abdomen in a rat following injection of ATP (in 1 ml of physiological saline) in these areas. I) 1000 γ; II) 500 γ; III) 250 γ; IV) 25 γ; V) physiological saline (absence of reaction).

Fig. 2. Deposition of trypan blue (1 ml of a 1% solution injected intraperitoneally) in areas of the skin of the abdomen after injection of ATP and MAP into these areas. I) 250 γ ATP; II) 500 γ ATP; III) 250 γ MAP; IV) 500 γ MAP; V) site of injection of physiological saline. The deposition of the dye was more intensive at the sites of injection of ATP.

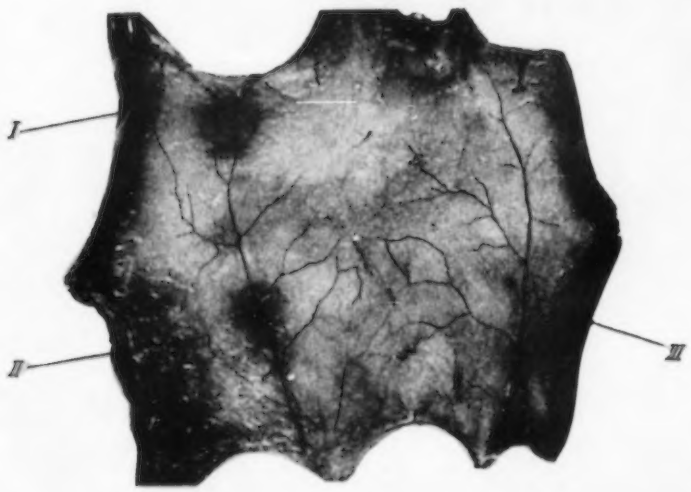

Fig. 3. Deposition of trypan blue (1 ml of a 1% solution injected intraperitoneally) in areas of the skin after injection therein of I) 500 γ ATP; II) 250 γ ATP in 0.1 ml of physiological saline. The point on the right – the site of injection of 0.1 ml of physiological saline (marked).

the action of the same dose of adenylic acid, staining appeared later and was less intensive (Fig. 2). In the control areas of skin, into which physiological saline was injected, sometimes no deposition of dye was found, and only in the center, at the site of puncture, did a blue spot develop. In some experiments the skin was also examined from the side of the dermis, where the reaction was particularly clearly expressed (Fig. 3).

It was not always possible to establish any definite relationship between the intensity of staining and the dose of the substance injected. At the same time, a clear relationship was found between the intensity of staining and the quantity of trypan blue injected. It thus became obvious that it was necessary to take account of the minimum quantity of dye required for demonstration of the effect of staining the tissue.

The investigations showed that ATP has a stronger effect on the permeability of the vessels than adenylic acid, which plays an important part in the stimulation of phagocytosis. The importance of adenine nucleotides and of decomposition products of nucleic acids in increasing the vascular permeability has also been demonstrated by other researchers [8, 12, 13].

SUMMARY

The effect of adenine nucleotides in causing a vascular permeability is discussed. As established in experiments on white rats, ATP causes a clear increase of vascular permeability in the skin of rats. This is detectable with the aid of vital trypan blue staining following intraperitoneal injection of the stain. The action of adenylic acid on the vascular permeability is less pronounced than that of ATP.

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SOME ASPECTS OF THE PATHOGENESIS OF EDEMA OF THE LUNGS DUE TO SILVER NITRATE

Communication III. The Mechanism of Death of Experimental Animals

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 70-75, August , 1960
Original article submitted May 15, 1959

It follows from the findings of A. M. Kotovshchikov [3], who made a special study of the mechanism of development of edema of the lungs following injections of $AgNO_3$, and also from several other investigations [1, 2, 4], that after injection of $AgNO_3$ severe circulatory disturbances are often observed in addition to edema. Our researches showed that the circulatory disturbances after intravenous injections of silver are caused by spasm of the vessels of the lesser circulation. The circulatory changes, however, are not the principal mechanism of the pulmonary edema, but constitute rather a process running parallel to the edema.

Accordingly, it was considered of interest to ascertain the main cause of death after injection of $AgNO_3$ – the edema of the lungs or the circulatory disturbances arising as the direct result of the action of this compound. The present communication is devoted to the elucidation of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on adult dogs of both sexes, which received injections of morphine hydrochloride in a dose of 0.01 g/kg before the beginning of the experiment. In order to assess the disturbances of the circulation, recordings were made of the arterial pressure and the pressure on the arterial side of the pulmonary circulation. As an index of the circulatory disturbances, we chose the time of onset of a severe, irreversible fall in the arterial pressure. The intensity of the edema was estimated by the ratio between the weight of the lungs in their natural state and the weight of the dry residue of the lung tissue. The arterial oxygen saturation was also recorded by means of a photoelectric oxyhemometer, and tracings were also made of the respiratory movements of the chest. The moment of death of the animals was obtained from the cessation of the tracing of respiration and of the activity of the heart. An aqueous solution of $AgNO_3$ was injected intravenously in a dose of 3.2 mg/kg in 0.4% solution into 14 animals, and of 32 mg/kg in a 4% solution into 5 animals. In the analysis of the results, we calculated the arithmetic mean ($M \pm m$) and the coefficients of correlation ($r \pm m_r$). The results were regarded as significant if the following inequality was observed:

$$\frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}} > 3 \text{ or } \frac{r}{m_r} > 3.$$

EXPERIMENTAL RESULTS

The circulatory disturbances in the experiments in which $AgNO_3$ was injected in a dose of 3.2 mg/kg were uniform in type, and consisted in particular of the inadequate influx of blood into the systemic circulation. After definite intervals of time from the moment of injection of the preparation, the arterial pressure began to fall (Table 1).

A direct, statistically significant connection was found between the time of development of a persistent fall of blood pressure, indicating the onset of serious disturbances of the systemic circulation (circulatory anoxia), and the length of survival of the animals (see Table 1).

Observations on the oxygen saturation of the arterial blood after injection of $AgNO_3$ showed that, initially, the saturation was maintained at figures close to the original level, after which a gradual fall took place. The degree of fall of the oxygen saturation of the blood varied in different experiments, and was definitely related to the length of survival of the animals after injection of the $AgNO_3$.

It will be seen from Table 1 that a direct, statistically significant correlation was present between the degree of the fall in the oxygen saturation of the arterial blood, due to the presence or absence of anoxic anoxia, and the length of survival of the animals after the injection of $AgNO_3$. In other words, the duration of survival of the animals was not decreased in those cases

TABLE 1. Certain Indices Characterizing the Injection of $AgNO_3$ in a Dose of 3.2 mg/kg

Experiment	Time (min) between $AgNO_3$ injection and beginning of arterial pressure fall	Magnitude of O_2 saturation fall, arterial blood (conventional units)	Dry residue of lung tissue (in %)	Duration of animals' survival (min) after $AgNO_3$ injection
	I	II	III	IV
1	4.5	7.4	14.88	12
2	2.0	8.5	14.05	13
3	7.0	9.2	12.37	16
4	6.5	8.6	15.05	22
5	12.0	10.0	13.70	22
6	10.0	11.2	12.80	25
7	2.0	15.8	9.90	25
8	9.0	14.2	15.60	25
9	14.0	12.2	14.06	27
10	2.0	9.6	13.11	27
11	17.0	11.4	10.55	28
12	14.0	10.3	13.24	28
13	15.0	12.4	10.87	29
14	13.0	12.6	12.43	30
Arithmetric mean (M ± m)	9.1±1.38	10.9±0.63	13.00±0.46	23.5±1.57

Note. Coefficient of correlation for I and IV $r_1 = 0.65 \pm 0.17$; for II and IV $r_2 = 0.64 \pm 0.016$; for III and IV $r_3 = -0.36 \pm 0.23$; for I and II $r_4 = 0.24 \pm 0.25$.

in which the oxygen content of the arterial blood was low.

From the post-mortem findings, the changes in the lungs formed a perfectly uniform pattern, characterized by marked edema and severe hyperemia.

The data shown in Table 1 demonstrate that no regular connection exists between the duration of survival of the animals after injection of $AgNO_3$ and the intensity of the edema. An inverse, but not statistically significant, correlation was found between the duration of survival of the animals and the dry residue of lung tissue. No causal connection was established in our experiments between the intensity of the edema and death of the animal.

A tenfold increase in the dose of the $AgNO_3$ injected (32 mg/kg) led to a still more rapid disturbance of the systemic circulation, by comparison with the experiments in which a dose of 3.2 mg/kg was given (the difference was statistically significant; cf. Tables 1 and 2).

The kymograms show that when the larger dose of the preparation was injected, the fall of arterial pressure came on quickly (Fig. 1); with the smaller dose (Fig. 2) no significant changes in the arterial pressure had taken place after the same interval of time.

The survival period of the dogs after injection of $AgNO_3$ and the ratio between the weight of the lungs and of their dry residue differed (statistically significantly) from the corresponding indices in the previous series of experiments (cf. Tables 1 and 2). The oxygen saturation of the arterial blood was also slightly higher (the difference was not statistically significant).

As it was pointed out above, after the intravenous injection of $AgNO_3$ two fundamentally independent pathological processes develop: edema of the lungs and circulatory disturbances caused by spasm of the vessels of the lesser circulation. Both the edema of the lungs and the circulatory disorders could have been the cause of death. In the first case, it is a matter of death as the result of anoxia of an anoxic character, in the second, of anoxia of a circulatory character; in these conditions the inadequate oxygen saturation of the arterial blood could have a significant effect on the hemodynamics, but the circulatory disturbances themselves did not essentially affect the process of saturation of the blood in the lungs with oxygen.

The inverse correlation between the duration of survival of the animals and the oxygen saturation of the arterial blood is undoubtedly evidence against the fundamental role of anoxic anoxia itself in the mechanism of death of the experimental animals. The hemodynamic disturbances of the systemic circulation, arising in consequence of anoxic anoxia, also could not be a fundamental link in the mechanism of death, since a

direct, but statistically insignificant, correlation was found between the time of development of an uncompensated fall in the arterial pressure and the magnitude of the fall in the oxygen saturation of the blood; this demonstrates the absence in this case of a relationship between the onset of the fall in the arterial pressure and the oxygen content of the arterial blood. It might have been assumed that a comparatively small fall in the percentage of oxygen saturation of the arterial blood leads rapidly to considerable circulatory disturbances. Experiments in which the more severe manifestations of anoxic anoxia failed to cause such a rapid disturbance of the circulation, however, are contrary to the acceptance of such a mechanism of the circulatory disturbances.

The rejection of a leading role of anoxic anoxia in the mechanism of death still does not imply acceptance of circulatory anoxia, resulting directly from disturb-

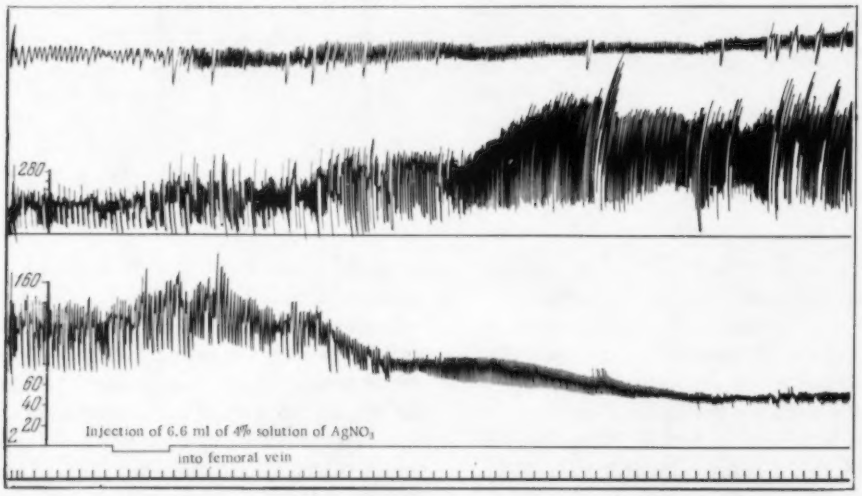

Fig. 1. The effect of intravenous injection of silver nitrate solution (6.6 ml of a 4% solution) on the respiration and blood pressure. Experiment No. 19. Male dog weighing 8.2 kg. Dose of $AgNO_3$ injected, 32 mg/kg. Significance of the curves (from above down): respiratory movements of the chest; pressure inside the right ventricle of the heart (scale in mm H_2O); pressure in the common carotid artery (scale in mm Hg); signal line; time marker – 5 seconds.

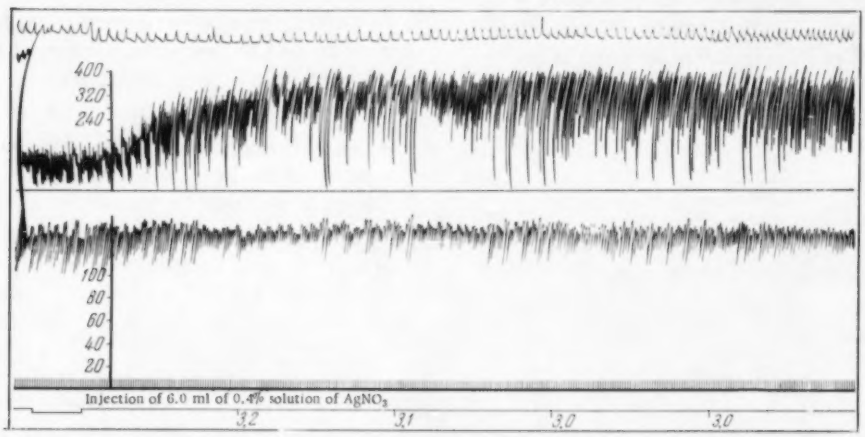

Fig. 2. The effect of intravenous injection of silver nitrate solution (6 ml of 0.4% solution) on the respiration and blood pressure. Experiment No. 4. Male dog weighing 7.5 kg. Dose of preparation injected, 3.2 mg/kg. Significance of the curves (from above down): respiratory movements of the chest; pressure inside the right ventricle of the heart (scale in mm H_2O); pressure in the common carotid artery (scale in mm Hg); time marker – 1 second; signal line.

TABLE 2. Certain Indices Appertaining to the Injection of $AgNO_3$ in a Dose of 32 mg/kg

Experiment	Time (in minutes) elapsing between moment of injection of $AgNO_3$ and beginning of fall in arterial pressure	Magnitude of fall in oxygen saturation of arterial blood (in conventional units)	Dry residue of lung tissue (in %)	Duration of survival of animals (in minutes) after injection of $AgNO_3$
15	1	6.4	18.87	9
16	2	7.6	21.07	10
17	4	8.8	16.55	12
18	3	8.0	17.98	13
19	3	14.0	17.51	18
Arithmetic mean (M ± m)	2.6 ± 0.41	8.95 ± 1.32	18.4 ± 0.77	12.4 ± 1.56

ance of the circulation by the action of $AgNO_3$ on the vessels of the lesser circulation, as the principal cause of death. However, the presence of a direct connection between the onset of the circulatory disturbances and the duration of survival of the animals makes such a point of view appear highly probable.

Injection of a large dose of $AgNO_3$ (32 mg/kg) led to the earlier death of the animals in association with very rapidly developing circulatory disturbances and a lower intensity of edema; this also is evidence against the leading role of edema of the lungs in the mechanism of death, and directly demonstrates the importance of the circulatory disturbances in this respect.

When the dose of $AgNO_3$ is increased, the relative importance of the circulatory disturbances caused directly by the silver nitrate in the mechanism of death of the animals also increases considerably. The principal pathogenetic mechanism responsible for death of the experimental animals is thus the circulatory anoxia associated with the action of $AgNO_3$ on the circulatory system.

SUMMARY

The mechanism of death was studied while investigating the pathogenesis of pulmonary edema induced by intravenous injections of silver nitrate. Hemodynamic and some other indices were recorded. A direct relationship exists between the life span following $AgNO_3$ injection and hemodynamic disturbances. There was an inverse correlation between the length of life and the oxygen content in the arterial blood. Observations presented show that the death of the animals was caused chiefly by the circulatory hypoxia induced by primary changes in the circulatory system resulting directly from the action of silver nitrate.

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THE GENESIS OF PHYSIOLOGICAL IMMATURITY AND ITS RELATIONSHIP TO KETONURIA IN THE MOTHER DURING PREGNANCY

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 75-80, August, 1960
Original article submitted July 1, 1959

Numerous investigations [1-6] have shown that the normal intrauterine development of the fetus is controlled by the regulating influence of a gestational dominant, developing at the beginning of pregnancy.

The regulating action of the gestational dominant determines the pituitary's gonadotropic function, specifically for pregnancy, and thereby the hormonal function of the corpus luteum. The latter makes possible the implantation and the formation of a physiologically efficient placenta, ensuring the normal development of the embryo and fetus. I. A. Arshavskii [2, 3, 4, 5] drew attention to the fact that inhibition of the gestational dominant, caused by the formation (at suitable times during pregnancy) of a new dominant, in association with the action of a variety of pathogenic agents, leads to disturbance of the normal formation of the placenta and also to metabolic changes, characterized by a significant shift toward acidosis.

Reports in the literature [8, 10, 12, 16, 20] testify to the fact that the most essential component of the acidotic state of the blood in pregnant females is incompletely oxidized products of the breakdown of lipids, namely, ketone bodies (β-hydroxybutyric and acetoacetic acids, and acetone). A factor of secondary importance is the formation of lactic acid. The problem of the transfer of ketone bodies from the maternal blood to the fetal blood (in abnormal pregnancy) has not yet been solved, on account of the impossibility of estimation of ketone bodies in the fetal blood, whether in man or laboratory animals (owing to the difficulty of obtaining plasma or serum in sufficient quantities, especially in the early stages of pregnancy). For these purposes it was necessary to choose an animal in which the fetus is characterized by the presence of a large enough allantois. Accordingly, the fetus of cattle was selected as test object.

The aim of the present investigation was to study the possibility of the transfer of ketone bodies from the maternal blood to the fetal blood, the development of the placenta and the characteristics of the fetus in relation to the presence or absence of ketonuria in the mother during pregnancy, and the state of physiological maturity of newly born calves in relation to the presence or absence of ketonuria in the mother.

EXPERIMENTAL METHOD

The material to be investigated was collected directly from the Moscow Mikoyan Meat Combine and from the Voskresenskoe and Ramenskoe collective farms in the Moscow region.

At the meat combine, the fetuses, placenta, and corpora lutea were carefully extracted from cows slaughtered at different periods of pregnancy. In each case the urinary bladder was removed from the cow for collection of the urine, and samples of the allantoic and amniotic fluids were collected from the fetuses. All these fluids were subjected to qualitative analysis for ketone bodies by Roser's test [19]. Attention was paid to the rate of development of the reaction – instantaneous, or developing after not more than 3-5 minutes, by which the degree of ketonuria could be judged.

In the collective farms, newly born calves and their placentas were studied. A short time after their delivery from the mother, urine was collected, and during calving the allantoic and amniotic fluids were obtained (also for analysis for ketone bodies).

The investigation of the fetuses and their placentas was carried out in the following order: the fetuses were weighed, and their length from the occiput to the base of the tail, and the length of the tail, were measured. The external morphological condition of the fetuses was assessed from the following signs: ossification of various parts of the skeleton, the presence and distribution of the hair cover, the length of the limbs, opening of the palpebral fissure and the presence of eyelashes and teeth, and descent of the testicles into the scrotum.

The age of the fetus was determined from these findings. For this purpose we used the indices recommended by G. A. Shmidt [14] and A. P. Studentsov [12]. Since the placenta in cattle consists of separate cotyledons (placenta multiplex), all the fetal placentas were counted, weighed, and measured. The placentas were subdivided into three groups in accordance with their size: large, medium, and small. Within the limits of each group, the arithmetic-mean surface area of the individual placenta was calculated, and the total surface area deduced. The total weight of the placentas and the weights of the three groups were determined. The material thus obtained was distributed in accordance with age groups. Within the limits of each age group, we compared the results obtained in the mothers whose urine contained ketone bodies with those in the mothers in whose urine ketone bodies were not detected. Altogether 45 fetuses and 15 calves were investigated.

EXPERIMENTAL RESULTS

Of the 60 pregnant cows investigated, only in 29 was ketonuria not found (normal pregnancy); in the remaining 31 ketonuria was present. When no ketone bodies were found in the mother's urine, they were also absent from the allantoic and amniotic fluids of the fetuses and the newly born calves. The presence of ketone bodies in the mother's urine was accompanied by their appearance in the allantoic fluid, but they were not present in the amniotic fluid. Thus, in the presence of ketonuria in the mother, ketone bodies pass from the maternal blood to the fetal blood, and thence, through the kidneys, into the allantoic fluid.

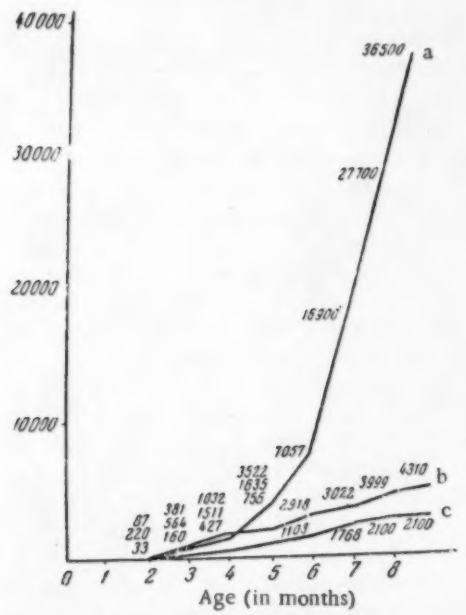

Fig. 1. Changes in the weight of the fetus, and the weight and surface area of the placenta at different periods of normal pregnancy. a) Weight of fetus; b) surface area of placenta; c) weight of placenta.

Let us consider the findings relating to the placentas and the weights of the fetuses obtained from mothers in which ketonuria was not present. In our investigations the earliest age of the fetuses corresponded to two months of intrauterine development (duration of pregnancy in cows is on the average 285 days).

In Fig. 1 are given the results showing the changes in the weight of the fetus (a), the total weight of the placenta (c), and the total surface area of the placenta (b) at different times during pregnancy. Between the end of the second month (87 g) and the end of the pregnancy (36,500 g), the weight of the fetuses increased roughly 420 times. Between the end of the second month of pregnancy (33 g) and the end of pregnancy (2100 g), the weight of the placentas increased only 64 times. The greatest increase in the body weight of the fetus took place during the last months of pregnancy. The greatest increase in the total weight of the placenta occurred during the first months (up to the 4th or 5th) of pregnancy, after which the further growth in the weight of the placenta followed an exponential curve. At the end of the second month of pregnancy, the number of cotyledons was 67; at the end of the third month, 71. Beginning with the fourth month, the number of cotyledons became more or less constant until the end of pregnancy, amounting on the average to 80-90. In isolated cases, over 100 cotyledons were found (as many as 140). The greatest portion of these cases occur in large and medium-sized placentas, and only very few in small placentas.

The same lack of correlation was thus found with respect to the fetuses of cattle, between the increasing dimensions of the total delineated area of the placental surface (which is judged by the weight of the placenta) and the increasing weight of the fetus, as has been established with respect to the fetuses of other mammals by Barcroft [15], Huggett and Hammond [19], and I. A. Arshavskii [1]. The researches of I. A. Arshavskii and his co-workers [6, 7, 11] showed that this lack of correlation during normal pregnancy is compensated by corresponding reactions of the developing fetus, namely, by the adaptive functions of its cardiovascular, respiratory, and musculoskeletal systems. In the cows in whose urine no ketone bodies were found, the corpus luteum of pregnancy acquires its greatest weight at the end of the third month, and in different cows its weight varies between 6.3 and 7.2 g. Starting with the second half of pregnancy a fall in the weight of the corpora lutea takes place, on account of their commencing involution. The subsequent repeated enlargement of the corpus luteum is known to be due to proliferation of connective tissue, supplanting the reducing lutein cells [13, 17].

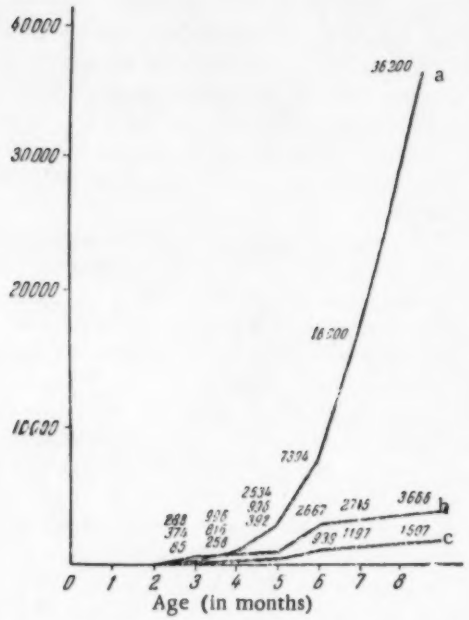

Fig. 2. Changes in the weight of the fetus and in the weight and surface area of the placenta at different times during pregnancy in the presence of ketonuria. a) Weight of fetus; b) surface area of placenta; c) weight of placenta. In these cases no fetuses less than 3 months old, or 7 months old or older, were investigated.

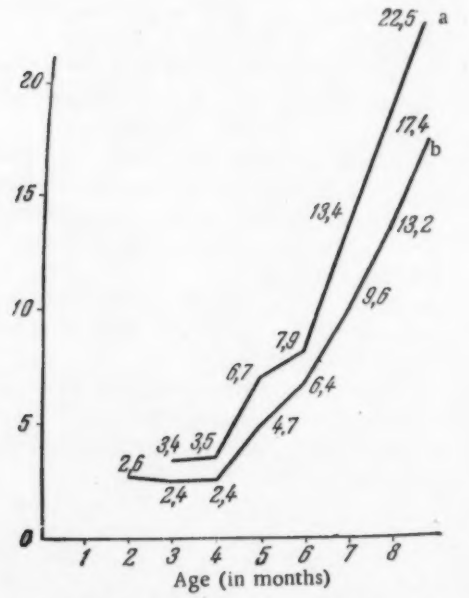

Fig. 3. Relationship between the weight of the fetus and placenta at different times during pregnancy. a) During pregnancy with the presence of ketonuria; b) during normal pregnancy.

The data shown in Fig. 2 show the change in the weight of the fetus (a), the total weight of the placenta (c), and the total surface area of the placenta (b) at different times during pregnancy in cows in whose urine ketone bodies were found. In the first place, attention is drawn to the fact that the weight and surface area of the placenta during all the months of intrauterine development have appreciably smaller values than the placenta in the previous group of cows. At the same time, the weight characteristics of the developing fetus differ only slightly from those of the fetuses of the previous group. Nevertheless, the fetuses in which ketonuria was demonstrated by such signs as the presence and distribution of the hair cover, the time of opening of the palpebral fissure, the time of appearance of the teeth, and the time of descent of the testicles into the scrotum, were characterized by clearly marked signs of physiological immaturity, by comparison with the fetuses of the previous group. When ketonuria was present during the first half of pregnancy, the corpora lutea were appreciably smaller in weight, varying between 4.5 and 5.5 g. Partial involution of the corpora lutea will naturally account for the less perfect placentation in the cows showing ketonuria. The less perfect placentation is shown, not so much by the number of cotyledons formed, as by their size. In cows with ketonuria, a considerable number of the placentas are of the small group, and comparatively few, of the large and medium-sized groups. The small size of the cotyledons corresponds to the small size of the caruncles, i.e., of the maternal part of the placenta.

In Fig. 3, are shown the results characterizing the changing relationship between the weight of the fetus and the weight of the placenta at the various months of intrauterine development in fetuses with the presence (a) and absence (b) of ketonuria. It can be seen that, at all stages of intrauterine development in fetuses with ketonuria, the weight of fetus per unit weight of placenta is far greater. This difference in the relative proportions shows that such fetuses are in much less suitable conditions as regards the supply of oxygen and nutrient substances than fetuses without ketonuria. As investigations carried out in our laboratory show, however, the physiological immaturity of fetuses with a less perfect placentation is created, not so much by their more limited supply of oxygen and nutrient substances, as by changes in their nerve centers due to acid metabolic products passing from the maternal to the fetal blood. It is evident that the ketone bodies play an important part in the induction of these changes in the fetal nerve centers.

We observed one special case in which, in the presence of a high degree of ketonuria and concentration of ketone bodies in the allantoic fluid (instantaneous reaction), the placenta began to undergo complete absorption, and death of the fetus took place at the age of about four months. The corpus luteum in this case was in a stage of almost complete involution.

Newly born calves were investigated during the course of one month (January, 1957). All the cases of ketonuria were observed in one of the collective farms (Ramenskoe) where not only was it possible to take note of this condition, but also, in contrast to the observations at the meat combine, more detailed information was available on the feeding conditions and general care of the animals, and on the physiological state of the newly born calves.

It is clear from the findings described above that newly born animals, developing in the presence of ketonuria in the mother, are smaller in weight and in the surface area of the placenta at each month of intrauterine development than newly born animals with a normal pregnancy. They are smaller in weight on the average by 493 g, and in surface area by 644 cm^2. The weight of the newly born animals is roughly the same in the two groups, and this naturally makes a much worse correlation between the weight of placenta and fetus (see Fig. 3). Newly born animals from mothers with ketonuria are characterized by lower indices of the heart rate, the respiration rate, and the body temperature, and also by a considerably weakened and sluggish sucking reflex.

All these calves developed a toxic dyspepsia within a few days of birth; three of nine calves investigated, from cows with ketonuria, died.

SUMMARY

The aim of this work was to investigate the peculiarities of placentation and characteristics of the fetus in relation to the presence or absence of ketonuria during pregnancy; the possible transfer of the ketone bodies from maternal into the fetal blood and the state of physiological maturity of the newborn with relation to the presence or absence of maternal ketonuria was also studied. The investigation was conducted on cows. The corpus luteum and placenta in cows with absence of ketonuria are characterized by normal development. The fetuses and newborn calves show signs of physiological maturity, i.e., specific features of their physiological functions correspond to their actual calendar age.

The presence of maternal ketonuria is associated with partial involution of the corpus luteum and with signs of imperfect placentation. In this case the ketone bodies pass from the maternal into the fetal blood.

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THE INFLAMMATORY REACTION IN CALVES DURING ONTOGENESIS

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 80-83, August, 1960
Original article submitted April 26, 1959

Reports in the literature [1, 2, 3, 5, 8, 9] have established that in early stages of phylo- and ontogenesis, the inflammatory reaction in the tissues is very feebly expressed on account of the refractoriness of the animal to the action of several stimuli.

In calves, in the process of ontogenesis, the inflammatory reaction has not been investigated. The present work is devoted to this subject.

EXPERIMENTAL METHOD

The inflammatory reaction was investigated in ten healthy cross-bred calves (red steppe breed, improved with Simmental). The age and weight of the animals at the time of the experiment are shown in Table 1. The animals were reared in unheated buildings, in individual stalls, on a diet to produce a mean daily gain in weight of 700 g. Until 10 days of age, the calves were given freshly obtained colostrum and milk four times a day ad lib., and subsequently three times a day; water was added after the first few days. After the fifth day, the calves were weaned on hay and concentrates.

The general body temperature and the skin temperature in 11 places previously freed from hair were determined in the animals by means of a radiometer. The hair was carefully clipped from the tip of the ear, where pigmentation was least, and this area was freed from fat. An injection of 0.1 ml of colorless turpentine was given subcutaneously into this area. By means of the radiometer, the temperature of this area was determined, and observations were conducted on the course of the inflammatory reaction, at first every minute, and later every 5 minutes, for 2 hours (in some animals for longer). On the following days the calves were investigated daily until complete recovery had taken place.

EXPERIMENTAL RESULTS

The results showing the changes in the temperature of the different areas of the body are summarized in Table 2.

It may be seen from these findings that the temperature of the "terminal" areas of the body of the newly born calf (nose, tip of the tail, hoof, ears), by virtue of their poikilothermism, was 20-21° below the body temperature. In calf No. 6, whose birth weight was 19 kg, the values of the skin temperature were lower than in calves of the age of one day and three days. This feature is directly connected with the backwardness of its embryonic maturation. In our previous investigations

TABLE 1. Characteristics of the Experimental Calves

Calf No.	Sex	Name	Live weight (in kg)		Age
			at birth	at subsequent investigations	
1	Female	Sazha	31	—	1 hr 30 min
2	Male	Venik	33	—	18 hr
3	Female	Ob'	23	—	24 hr
4	Male	Mart	35	—	68 hr
5	"	Plot	33	—	72 hr
6	"	Met	19	—	142 hr
7	Female	Manta	22	42.5	38 days
8	"	Sevryuga	25	51.0	44 days
9	Male	Fantik	39	93.0	59 days
10	"	Gusek	30	77.0	64 days

TABLE 2. Temperature of Different Parts of the Body in the Calves

Calf No.	Room temperature	Temperature of the skin of									Temperature		Body temp.
		nose	forehead	ear	crest	croup	tip of the tail	groin	chest in the heart region	wrist	hoof of the fore leg	hoof of the hind leg	
1	15°	18,0°	30,0°	23,0°	32,0°	30,6°	18,0°	32,0°	30,0°	22,0°	18,1°	19,0°	39,1°
2	15°	33,6°	34,0°	30,0°	35,6°	36,8°	33,0°	36,4°	36,2°	31,0°	23,4°	32,0°	38,5°
3	15°	31,2°	30,0°	26,0°	34,0°	35,8°	30,6°	34,4°	34,8°	32,6°	22,5°	21,0°	38,5°
4	15°	33,4°	31,0°	27,4°	31,0°	35,0°	24,2°	34,6°	34,2°	33,0°	24,2°	26,6°	39,4°
5	15°	32,6°	31,6°	25,6°	35,6°	35,2°	26,4°	37,8°	36,0°	31,4°	27,2°	20,8°	39,5°
6	16°	32,8°	34,0°	24,0°	35,0°	35,0°	32,2°	36,2°	32,4°	31,4°	26,0°	25,8°	38,6°
7	18°	32,0°	32,4°	32,0°	36,2°	34,2°	29,6°	36,4°	36,6°	34,2°	20,4°	21,1°	39,2°
8	18°	32,2°	34,0°	33,6°	35,4°	34,0°	31,6°	36,2°	35,4°	34,4°	27,4°	30,0°	39,0°
9	18°	33,4°	31,0°	28,0°	34,6°	34,0°	31,2°	37,4°	35,5°	34,2°	23,6°	19,0°	38,4°
10	18°	28,2°	30,8°	29,6°	34,2°	33,0°	19,8°	35,0°	34,2°	26,2°	18,6°	18,8°	38,7°

TABLE 3. Temperature Changes in the Region of the Inflammation

Calf No.	Time (in min) of investigation of skin temperature after injection of turpentine														Body temperature	
	normal	1	5	10	15	20	25	30	40	50	60	70	100	200	before expt.	at end of expt.
	Skin temperature															
1	23,0°	29,0°	32,2°	34,4°	34,4°	34,6°	34,4°	34,4	34,0°	33,6°	33,6°	—	—	—	39,1°	39,3°
2	30,0°	33,6°	35,4°	35,4°	—	35,8°	—	35,2	34,4°	34,4°	34,2°	34,8°	34,6°	33,2°	38,5°	38,5°
3	26,0°	32,4°	32,6°	34,6°	34,8°	35,0°	35,1°	35,1°	35,2°	35,2°	35,0°	—	35,0°	—	38,5°	38,7°
4	27,4°	35,2°	35,4°	35,2°	—	36,0°	—	36,2	36,3°	36,4°	36,4°	34,4°	32,2°	—	39,4°	39,5°
5	25,6°	35,4°	35,6°	35,4°	—	35,6°	—	36,0	36,2°	35,8°	35,6°	35,6°	34,2°	33,8°	39,5°	39,3°
6	21,0°	33,0°	34,2°	34,8°	—	35,2°	—	35,0	35,0°	35,2°	34,4°	34,6°	33,2°	—	38,9°	39,0°
7	32,0°	34,2°	35,4°	35,2°	35,4°	—	35,2°	—	35,4°	35,2°	35,6°	35,8°	—	—	39,2°	39,8°
8	33,6°	35,0°	35,8°	35,2°	35,4°	35,2°	36,2°	35,8	35,6°	35,8°	36,2°	—	—	—	39,0°	39,6°
9	28,0°	35,2°	35,6°	35,4°	35,6°	36,1°	35,9°	36,0	35,8°	35,8°	36,0°	36,2°	—	—	38,4°	39,4°
10	29,6°	35,2°	35,4°	35,4°	35,8°	36,2°	35,8°	36,2	36,2°	35,8°	36,0°	35,8°	—	—	38,7°	39,3°

[7], and also in work by other researchers [4, 6], it has been shown that the duration of poikilothermism in newly born mammals is dependent on the prenatal development.

At the site of injection of the turpentine into the experimental calves, an increase in temperature and the development of an inflammatory lesion were observed. Figures showing the temperature changes are given in Table 3.

The maximum skin temperature at the site of turpentine injection into the calves was noted at different times. In a newly born calf (No. 1), in calf No. 2, aged 18 hours, in the bull-calf Met (No. 6), and calf No. 10, a rise of temperature was observed at the same time. In the other calves the apogee of the thermal reaction occurred at different times.

When the temperature of the inflammatory area was compared with the normal temperature (with the temperature before injection of turpentine), it was found that the highest rise was present in calves Nos. 1 and 6, i.e., in the newly born calf and the premature, underdeveloped animal.

In calves Nos. 1, 3, and 6 the lowest temperature at the site of injection of the turpentine was observed. In calves Nos. 2, 4, and 5 the temperature was higher. These animals were of a considerable live weight at birth. In the older calves – Nos. 7, 8, 9, and 10 – the temperature of the inflammatory focus was very close to the temperature of the inflammatory foci in the calves Nos. 2, 4, and 5. At the site of injection of the turpentine into the younger calves, edema (puffiness) and other signs of inflammation developed around the needle puncture. In the course of its development the edema increased, and an abundant discharge of exudate took place, which was measured by counting the drops of exudate discharged per minute.

In the older calves the inflammatory reaction was imperceptible, the ear remained mobile, and redness and tenderness were absent.

In calves Nos. 1-6 a violent vascular reaction developed at the site of inflammation, accompanied by increasing edema and an abundant exudation. The inflammatory area was increased four- to sixfold in thickness, erythema was slight, tenderness insignificant, but the mobility of the ear was restricted.

When the temperature figures and the clinical findings were compared, it became obvious that in calves Nos. 1-6 the body temperature was not increased. Apparently turpentine does not cause a pyrogenic reaction in animals of this age.

Further investigations showed that in calves during the first days of life, profound alterative changes and deformation of the ear arose at the site of the inflammatory lesion. In the older calves the inflammatory reaction was mild and did not lead to visible disturbances of the underlying and surrounding tissues.

SUMMARY

The development of an experimentally induced inflammatory reaction was studied in newborn calves, during the first hours after birth ($1\frac{1}{2}$-142 hours), 38-42 days. In newborn calves the temperature of definite areas of the skin was lower (by 20-21°C) than the body temperature, and was subject to more pronounced fluctuations, than in the older ones.

At the site of turpentine injection (the agent causing inflammatory reaction) the temperature was two degrees lower in the newborn calves than in the older ones, whereas the vascular reaction was more active. The inflammatory process in the newborn, is more prolonged than in the older calves, and terminates with considerable changes and deformation of the tissue. No visible changes were observed in the tissue of older calves at the site of inflammation.

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PUPILLARY REACTIONS IN RABBITS EXPOSED TO SMALL DOSES OF Sr^{90}

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(Presented by Active Member AMN SSSR V. V. Zakusov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 83-86, August, 1960
Original article submitted July 6, 1959

An important consequence of exposure of an animal to radioactive substances is a change in its reaction to drugs [2]. Despite its obvious current importance, insufficient attention has been paid to this problem.

In this communication we shall deal with the changes in the pupillary reactions, especially to pharmacological agents, after exposure of rabbits to strontium 90. Changes in the pupils during chronic exposure to ionizing radiation, and, more especially, the behavior of the pupillary reactions, are not discussed in the literature.

EXPERIMENTAL METHOD

Experiments were conducted on ten rabbits which received subcutaneous injections of strontium 90 in a dose of 5 μC/kg/day for a period of $2\frac{1}{2}$ months, and also on ten control rabbits. Most animals showed initial signs of chronic radiation sickness: a fall in the leucocyte count (to 4000-5000 per mm^3 blood) and an increase in the permeability of the skin vessels by 10-20% compared with the initial level, as shown by the results of the fluorescein test. In order to record the size of the pupils, a Zenith-S camera with intermediate rings Nos. 2, 3, and 4 and an objective set at 0.8 m was used (Fig. 1). The apparatus was mounted on a stand with the pentagonal prism below. A micrometer scale with divisions of 0.1 mm was inscribed directly on the opaque glass of the apparatus. The image of the eye was superimposed upon the scale on the opaque glass, and the experimenter was able to observe these with a magnification of five times, on account of the eyepiece of the apparatus. The size of the pupils (the lesser diameter) was measured directly during the experiment on the scale; the accuracy of measurement was verified after development of the film. The light source (a 75 w lamp) was placed at a distance of 30 cm from the rabbit's eye at an angle of 30° to the axis of its body. The intensity of illumination was verified by a photoelectric method.

In order to investigate the pupillary reactions, six pharmacological preparations were used, possessing different mechanisms of action on the pupils: a narcotic (morphine), ganglionic preparations (nicotine and tetraethylammonium) and also drugs acting mainly on the peripheral nerve endings (eserine, pilocarpine, homatropine). All these drugs were used in nontoxic doses, which caused no disturbances of the animals' general condition. With each drug from 6 to 10 experiments were carried out on control rabbits and animals treated with radiostrontium (the doses and methods of administration are given in the table). Experiments were performed at intervals of not less than three days.

EXPERIMENTAL RESULTS

On the basis of nearly 200 measurements of the size of the pupils, it was found that the pupillary diameter in the treated rabbits, when the eye was illuminated with 3000 lux, averaged 5.0 ± 0.1 mm (here and subsequently, the first number indicates the arithmetic mean, and the second the mean square deviation). In healthy animals, under the same conditions, the pupillary diameter was slightly smaller, namely, 4.6 ± 0.1 mm. With an illumination of 1000 lux, the size of the pupils in the treated and normal animals was the same, namely, 5.5 ± 0.1 mm. The reaction of the pupils to light was thus weakened in the experimental animals, although only slightly.

The results of the investigation of the pupillary reactions to the administration of pharmacological agents are shown in the table.

By way of illustration of the experiments, we give examples of the individual pupillary reactions in the

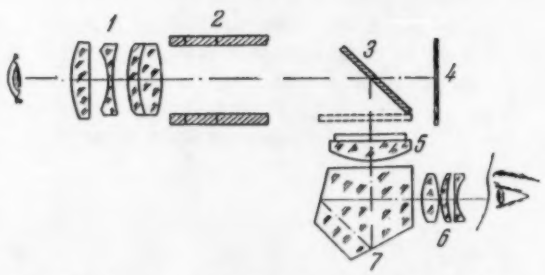

Fig. 1. Scheme of use of the Zenith-S camera for investigation of the pupillary reactions. 1) Objective; 2) intermediate rings; 3) mirror; 4) photographic film; 5) scale; 6) eyepiece; 7) pentagonal prism.

Pupillary Reactions in Rabbits Treated with Strontium 90 and in Controls

Drug	Dose and method of administration	Change in pupillary diameter (in mm)		Character of reaction
		experiment	control	
Morphine hydrochloride	0.2 ml/kg of 1% solution intravenously	1.0 ± 0.1	0.4 ± 0.1	Miosis
Nicotine	0.05 ml/kg of 0.1% solution intravenously	1.1 ± 0.2	0.4 ± 0.2	"
Tetraethylammonium iodide	0.25 ml/kg of 10% solution intravenously	2.5 ± 0.2	1.6 ± 0.1	Mydriasis
Eserine salicylate	2 drops of a 1% solution into conjunctival sac	3.0 ± 0.2	2.3 ± 0.2	Miosis
Pilocarpine hydrochloride	2 drops of a 1% solution into conjunctival sac	1.8 ± 0.1	1.7 ± 0.2	"
Homatropine hydrobromide	2 drops of a 1% solution into conjunctival sac	3.2 ± 0.3	3.2 ± 0.2	Mydriasis

experimental rabbits and compare them with the controls (Fig. 2).

Immediately after injection of morphine into the rabbits, we observed constriction of the pupils, and its intensity was 2.5 times greater in the experimental than in the control animals. The miotic action of morphine is due to excitation of the oculomotor nerve center. This effect is most marked when morphine is given, by comparison with other narcotics [3]; it may possibly develop secondarily as the result of the action of morphine on the higher divisions of the brain, which control the center of the oculomotor nerves [1]. It is interesting that in our experiments, when fractional doses of narcotics (urethan and hexobarbital) were given, it was shown that the pupillary reaction to morphine was hardly affected by variation in the depth of narcosis; but in the last stage of narcosis, when profound depression and arrest of breathing and severe bradycardia were found, it was weakened and later produced no effect, showing depression of the medullary centers. The direct action of morphine on the oculomotor center cannot therefore be rejected. These experiments demonstrate the considerable increase in the sensitivity of this center to the action of morphine after exposure to strontium 90.

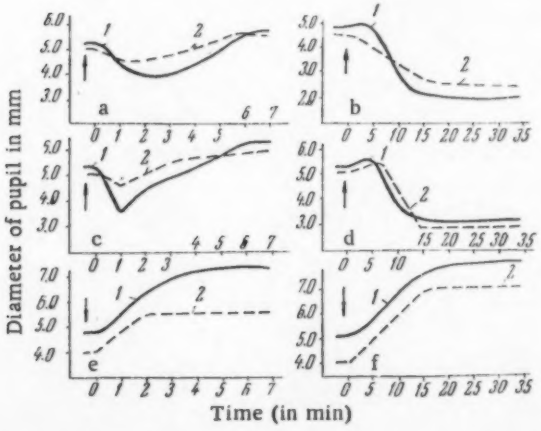

Fig. 2 Pupillary reactions to pharmacological agents in rabbits treated with strontium 90 (1) and in control rabbits (2). a) Morphine; b) eserine; c) nicotine; d) pilocarpine; e) tetraethylammonium; f) homatropine.

Injection of nicotine caused a transient constriction of the pupils, and the degree of miosis in the treated rabbits was almost three times greater than in the controls. Constriction of the pupils in response to nicotine is due mainly to its ganglionic action. Nicotine causes miosis by excitation of the nicotinelike cholinergic systems of the parasympathetic ganglia (in this case, the ciliary ganglion). We know from the literature that ganglion-blocking substances dilate the pupils. This property is fully shared by tetraethylammonium. As a result of the blocking of the parasympathetic and sympathetic (cervical) ganglia, dilatation of the pupils occurs as a result of the predominance of the dilator pupillae over the sphincter. In rabbits exposed to strontium 90, after administration of tetraethylammonium, the pupil was more strongly dilated than in the control animals. The results of these experiments indicate an increase in the sensitivity of the vegetative ganglia to the influence of ganglionic drugs.

Eserine, when instilled into the conjunctival sac, constricts the pupil because it is an anticholinesterase drug. As the table clearly shows, the miosis was rather more strongly expressed in the experimental rabbits than in the controls. In addition to its local action on the pupil, the resorptive influence of eserine must be borne in mind; it is revealed by the development of a profuse salivation. The miosis may therefore be dependent, not

only on the local action of eserine on the muscarine-like cholinergic receptors, but also on the nicotinelike cholinergic receptors of the ciliary ganglion. We found no significant difference between the miotic action of pilocarpine in the experimental and control rabbits. The same remark applies to the results of the experiments with homatropine, which causes mydriasis. The action of these two preparations is explained by their influence on the peripheral muscarinelike cholinergic systems. The sensitivity of these systems to pilocarpine and homatropine in the experimental rabbits was thus not changed. The more marked miotic action of eserine in the affected animals must evidently be attributed to an increase in the sensitivity of the nicotinelike cholinergic systems.

The increase in sensitivity to drugs with differences in their selective action cannot yet be given a detailed explanation. This fact is evidently caused by changes in different divisions of the nervous system (asthenization, alteration in the level of excitation, phasic conditions), and by disturbances of the enzyme and mediator (especially acetylcholine) metabolism. Increased permeability also leads to increased sensitivity to chemical substances [4]. The strengthening of the miotic action of drugs acting on the central nervous system and the vegetative ganglia may also be to some extent dependent on the weakening of the reaction to light in the experimental rabbits, for the initial diameter of the pupils when the experiments were carried out was slightly larger than that of the control animals.

The results obtained confirm the value of the pupillary reactions in the investigation of the functional state of an animal in chronic experiments.

SUMMARY

Experiments were conducted on rabbits with the initial signs of chronic radiation sickness. The following changes were observed in the affected animals: decrease of pupillary reaction to light and intensification of pupillary reactions induced by pharmacological substances affecting the central nervous system and the ganglionic apparatus (morphine, nicotine, tetraethylammonium). Reaction of the pupils to pilocarpine and homatropine remained unchanged.

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THE EFFECT OF FASTING ON THE CHOLESTEROL METABOLISM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 87-89, August, 1960
Original article submitted October 3, 1959

The work of G. G. Samsonidze [4], S. V. Nedzvetskii and S. S. Dubova [3], Sugano and co-workers [7], and Bragdon and co-workers [5] has demonstrated the constant development of hypercholesteremia in fasting animals. Much doubt and controversy remains, however, on the question of the mechanism of this phenomenon. Since the liver plays an important part in cholesterol metabolism, we deemed it worth while to investigate the part played by this organ in the development of hypercholesteremia in fasting animals. In the present series of investigations, the following problems were studied: 1) the cholesterol content of the liver in normal conditions and during fasting; 2) the rate of incorporation of radioactive (1-C^{14}) acetate in the liver cholesterol in normal conditions and during fasting.

EXPERIMENTAL METHOD

Experiments were carried out on 18 rabbits (6 controls and 12 fasting animals). After fasting for different periods of time (6 and 12 hours), the rabbits received intraperitoneal injections of 2 ml of a solution of sodium acetate (1-C^{14}) in a dose of 50 μC/kg body weight. The rabbits were sacrificed by exsanguination (division of the major vessels of the neck) 4 hours after the moment of injection of the acetate. The liver was extracted as quickly as possible, frozen, and then minced. The cholesterol, extracted from the liver, was precipitated in the form of cholesterol digitonide. The activity of the latter was determined by means of an end-type counter on a "B2" apparatus. The total cholesterol content of the liver was determined by a modification of Sperry's method [6].

EXPERIMENTAL RESULTS

On analysis of the results presented in the table, the following conclusions emerge. Fasting had a very considerable effect on the total cholesterol content in the rabbits' livers. On the sixth day of starvation the cholesterol concentration in the liver exceeded that in the control animals by 60%, and on the 12th day, by 83%. After fasting for 24 days, the concentration of cholesterol in the liver was increased by approximately 150%.

The results of these investigations agree with those of other authors with respect to changes in the cholesterol content of the tissues. O. N. Abbakumova-Zepalova [2], for instance, found an increase in the cholesterol content of the muscles of fasting rats, K. V. Gordeeva [1] found an increased cholesterol concentration in the liver of dogs after fasting three days, and G. G. Samsonidze [4] found an increase in this compound in all the organs which he investigated, which included the liver. Fasting also has a strong influence on the synthesis of cholesterol in the liver. After injection of radioactive acetate, the relative activity of the liver cholesterol in the fasting rabbits in every experiment, without exception, was less than that in the controls. It may be seen from the table that the rate of incorporation of 1-C^{14} acetate in the liver cholesterol did not exceed 10-20% of that taking place in the rabbits which received a normal diet.

In our experiments, as was also observed in the researches of many other authors, fasting was accompanied by a sharp rise in the blood cholesterol. After 6 days of fasting, the total serum-cholesterol concentration exceeded the initial level, on the average, by 140%, and after 12 days, by 188%.

It should be pointed out that during investigation of dogs after fasting for 10 days, the changes in the blood cholesterol concentration were less marked than in rabbits. After administration of 1-C^{14} acetate to rabbits, radioactive cholesterol was found in the blood, although, as the figures in the table show, the relative activity of the serum cholesterol was very low, approximately $^1/_2$-$^1/_3$ that of the cholesterol extracted from the liver. The ratio between the relative activities of the liver and serum cholesterol (4 hours after injection of radioactive acetate) was roughly the same in the normal and fasting animals.

In this investigation we discovered facts outwardly contradictory in their meaning. On the one hand, during fasting a considerable increase in the cholesterol concentration in the liver and blood was found. On the other hand, the synthesis of cholesterol in the liver (in so far as this could be judged by the rate of incorporation of C^{14} acetate) was greatly retarded. The question

Content and Relative Activity (in imp/min/mg) of the Cholesterol of the Liver and Serum

Animal No.	Total cholesterol (in mg%)		Relative activity of cholesterol		Note
	liver	serum	liver	serum	
1	302	73	93	29	Normal diet
2	216	66	59	27	"
3	237	81	104	36	"
4	271	62	77	41	"
5	177	78	61	24	"
6	186	74	89	39	"
Mean	231	72	80	32	
7	400	126	21	12	6 days of fasting
8	381	181	17	7	"
9	321	174	32	14	"
10	411	153	20	14	"
11	366	214	15	9	"
12	343	201	19	12	"
Mean	370	174	20	10	
13	387	252	18	8	12 days of fasting
14	466	212	21	6	"
15	399	191	26	9	"
16	424	222	16	12	"
17	496	175	12	10	"
18	368	197	19	9	"
Mean	423	208	18	9	

arises: how is it possible to explain the fact that the cholesterol content in the liver and blood is increased during fasting? In an attempt to answer this question, certain authors [4] have suggested that the increase in the cholesterol concentration during fasting takes place as the result of new formation of the compound in the liver. As the results of our investigations show (in agreement with those of several other authors) cholesterol synthesis in the liver during fasting is not only not increased but, quite the contrary, is considerably diminished. It might have been expected that, in fasting, the synthesis of cholesterol is intensified in other organs, from which it is taken in the blood stream to the liver. In this case the relative activity of the cholesterol of the blood would have been higher than, or at least equal to, the relative activity of the cholesterol of the liver; but, as our results show, the converse was in fact true. This supposition is thus not borne out by the facts. It is possible that the increase in the cholesterol concentration in the blood and liver is the result of a decrease in its breakdown or excretion from the body during fasting, but this is a matter for further research.

SUMMARY

The author studied the effect of starvation upon the cholesterol content in the liver and blood, as well as on cholesterol synthesis in the liver. 1-C^{14} sodium acetate was injected intraperitoneally into rabbits (50 μC per kg of body weight). The animals were bled to death 4 hours after C^{14} administration. Cholesterol – C^{14} was isolated in the form of the digitonide. Starvation was connected with a considerable increase of cholesterol concentration in the blood and liver and with an acute reduction of the rate of cholesterol synthesis in the liver. The correlation between specific activities of the liver and blood serum cholesterol is about the same in normal and starving animals. The reduced chloresterol excretion and breakdown possibly causes a rise of its level in starving animals.

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THE EFFECT OF UNILATERAL DECORTICATION ON CALCIUM METABOLISM IN THE CENTRAL NERVOUS SYSTEM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 89-92, August, 1960
Original article submitted November 2, 1959

For many years (1934-1958), in Prof. É. A. Asratyan's [1] laboratory, various reflexes were investigated in dogs after extirpation of the cerebral cortex. In these investigations studies were made of the changes in the general functions of the body after total decortication, and in the functions of symmetrical organs after unilateral decortication. There is no doubt that all the disturbances after unilateral decortication are associated with definite changes in the functional state and activity of those nervous structures which are actually responsible for controlling the work of the corresponding organs, i.e., which carry out the reflex regulation of the body functions. On the basis of much research proving beyond doubt the close relationship between physiological and biochemical processes [4, 10, 14, and others], it would be reasonable to expect some form of disturbances in the chemical activity of the nervous centers after extirpation of the cortex of one hemisphere. It would also be reasonable to postulate changes in the relationship between the nervous tissue and various neurotropic elements – calcium, in particular.

The aim of the present research was to study the changes in the calcium metabolism in different areas of the central nervous system after unilateral decortication by means of the radioactive isotope Ca^{45}. We chose this indicator of the biochemical disturbances in the nervous system after decortication in view of the undoubted importance of the calcium ion in fundamental nervous processes [7, 8, 9, 11, 18, 19].

EXPERIMENTAL METHOD

Experiments were carried out on rabbits of both sexes, weighing from 1900 to 3000 g. In each experiment three rabbits were used: one after decortication, one after trephining, and the third as a control. All the animals were taken at the same time from the nursery and kept under identical conditions. The operations of decortication and trephining in all the experiments were performed on the right side. The interval between operation and experiment was 37-43 days. Three hours before the rabbits were sacrificed, all received intravenous injections of a solution of $Ca^{45}Cl_2$. Altogether, nine experiments were performed on 27 animals. The isotope was injected in a dose of 100 μC/kg body weight. Three hours after the injection of Ca^{45} the rabbits were sacrificed by means of air embolism. Weighed samples of the right and left halves of the diencephalon, the midbrain, the medulla, the cerebellum, and the spinal cord were transferred to porcelain crucibles and incinerated in a muffle furnace at a temperature of 800°. To the dry residue after incineration was added 0.2 ml HCl (1 : 1), and to this solution, distilled water to a volume of 1 ml. From the solution thus obtained two targets were prepared for counting, and to each of them 0.2 ml of this solution was applied.

The activity of each target was determined twice (for 4 minutes) with an end-type counter on a "B" apparatus. The number of impulses in a weighed sample of brain was calculated per g of fresh tissue. At the same time the activity of the serum was determined, having been obtained by means of a syringe from the heart before the animals were sacrificed.

EXPERIMENTAL RESULTS

The object of the research was to ascertain the effect of the operation of unilateral decortication on the calcium metabolism in the central nervous system by comparison with that in an animal after unilateral trephining and an intact animal. This was done by comparing the level of Ca^{45} in corresponding divisions of the brain and spinal cord of the animals of all three groups; the radioactivity of the right halves of the brain structures taken for the experiment from the intact rabbit was compared only with the radioactivity of the right halves from the trephined and decorticated rabbits, and this was done in the same way with respect to the radioactivity of the left halves of the corresponding divisions. Table 1 shows how the accumulation of Ca^{45} in the structures of the nervous system on either side was altered after the operations of unilateral trephining

TABLE 1. Ca^{45} Content (in imp/min/g) in Various Divisions of the Central Nervous System of Rabbits 3 Hours after Intravenous Injection of $Ca^{45}Cl_2$

Division of the CNS	Right (operated) side			Left (unoperated side)		
	decortication	trephining	control	decortication	trephining	control
Spinal cord	2 220±113	1 930±272	1 875±151	1 960±109	2 362±349	1 800±136
Medulla oblongata	3 032±155	2 118±132	2 258±158	2 728±194	2 505±241	2 241±109
Midbrain	2 368±132	2 034±130	2 173±159	2 160±90	2 033±134	2 097±174
Diencephalon	2 350±145	1 800±124	1 845±171	2 113±218	1 807±235	1 877±112
Cerebellum	2 531±307	1 984±149	2 015±230	2 118±325	2 102±235	2 045±150

TABLE 2. Ratio of Radioactivity of the Right Side of Divisions of the Central Nervous System to Radioactivity of the Left Side in a Rabbit 3 Hours after Intravenous Injection of the Radioactive Isotope of Calcium

Division of the CNS	Decortication	Trephining	Control
Spinal cord	1.13±0.090	1.02±0.074	0.99±0.080
Medulla oblongata	1.14±0.107	0.95±0.052	1.0 ±0.054
Midbrain	1.11±0.058	1.00±0.033	1.05±0.055
Diencephalon	1.33±0.087	1.0 ±0.05	0.99±0.073
Cerebellum	1.25±0.14	1.03±0.08	1.05±0.08

and decortication, by comparison with the initial level in the intact rabbit.

It is easy to observe that after the operation of decortication the Ca^{45} content increased both on the right (operated side) and on the left (unoperated side); the increase observed on the left, however, was much more weakly expressed. The operation of trephining was not followed by any lasting changes in the calcium metabolism. It can be seen from the results in Table 1 that there was no sharp difference between the various brain formations in their Ca^{45} content, if no account be taken of the fact that the medulla showed a tendency toward an increase, and the spinal cord a tendency toward a decrease, in the content of radioactive isotope from the mean level.

By means of a different treatment of the results obtained, however, it was possible to show how there was a considerable asymmetry in the accumulation of Ca^{45} within each division between the half lying on the side of the operation, and that on the unaffected side. For this purpose the Ca^{45} content of one side of each division of the brain was taken as unity, and the ratio between the total Ca^{45} of the opposite side and this quantity was calculated. It is clear that if no asymmetry exists within the limits of the brain structure under consideration, this ratio will be equal to unity, as was observed in the control rabbits. It may be seen from Table 2 that the operation of decortication disturbed the equilibrium existing before operation: the ratio between the Ca^{45} content of the operated side and that on the unoperated side in all divisions was greater than unity – in the spinal cord it was 1.13, in the medulla 1.14, and so on. In the trephining experiments no statistically significant changes in the calcium metabolism were found. The different divisions of the central nervous system reacted differently to unilateral decortication. The most obvious changes were found in the diencephalon, followed by the cerebellum, the medulla, the spinal cord, and the midbrain.

As a result of these experiments it may be stated that unilateral extirpation of the cerebral cortex led to an increase in the accumulation of Ca^{45} in the subjacent divisions of the central nervous system, mainly concentrated on the side of operation. Changes in the calcium concentration are known to affect the working of the nervous system as a whole, both central and peripheral [12, 13, 15, and others]. Not without reason is the calcium system one of the most stable in the body [17].

In its turn, an increase in the calcium metabolism in the nervous system, in response to some influence acting upon it, is a part of the reaction of the nervous tissue to the agent concerned. The paralyzing, depressing action of the calcium ion, the properties of which have been widely employed in medical practice, and also the tendency of the nervous tissue to retain calcium in the brain during narcosis, suggest that a close relationship exists between calcium and the process of inhibition [6].

On the basis of these data and the results of our own experiments, we consider that the accumulation of Ca^{45} in the brain after unilateral decortication reflects the similarity between the process taking place during artificial or natural sleep, or depression, and the state in which the subjacent nervous centers are found after operation. The theoretical basis of this hypothesis is Pavlov's idea of protective inhibition, developed by É. A. Asratyan, who postulated that protective inhibition is a universal reaction of the nervous system in both functional and organic disorders.

The differences in the accumulation of Ca^{45} in the divisions of the central nervous system that were tested are regular, and are evidently due to their distinctive structural and biochemical features, which have developed as a result of long evolution. Only guesses may be made regarding the true role of the accumulation of Ca^{45} in inhibited structures. That this is a beneficial phenomenon is clear from the very meaning of the process accompanying the calcium ion. It is possible that the calcium ion, with its many functions, has yet another, connected in some manner with the beneficial aspect of the action of the inhibition process.

SUMMARY

The effect of unilateral decortication and trephining upon the calcium metabolism in the diencephalon, mesencephalon, medulla oblongata, cerebellum, and spinal cord was studied by a radioactive method. Experiments were conducted 40 days after the operation. The results of experiments demonstrated that decortication induces a rise of radiocalcium accumulation in these nervous formations, mainly concentrated at the site of operation. No statistically significant changes were revealed after trephining.

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THE CHARACTER OF THE ANTAGONISM BETWEEN DRUGS OF THE "PACHYCURARE" GROUP AND CERTAIN DEPOLARIZING AGENTS IN FROG RECTUS ABDOMINIS MUSCLE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 93-97, August, 1960
Original article submitted June 30, 1959

The idea that competitive antagonism with acetylcholine underlies the mechanism of action of the "pachycurare" drugs* is not based on any substantial number of investigations devoted primarily to the quantitative study of the antagonism between the pachycurare drugs and depolarizing agents. Observations show that the antagonism for acetylcholine displayed by pachycurare drugs (gallamine triethiodide [5], d-tubocurarine, C-toxiferine II, and others [8]) does not entirely fit into the framework of concepts of competitive antagonism between these substances.

The present investigation was concerned with a study of the character of the antagonism between pachycurare drugs (d-tubocurarine chloride, Paramyon, and Diplacine) and such depolarizing agents as acetylcholine and succinylcholine.

METHOD

Experiments were carried out on the rectus abdominis muscle of the frog (males of species R. esculenta). The muscle was suspended in a 100-ml beaker in an isotonic sodium chloride solution, under conditions of constant aeration. In the first series of experiments we determined the concentrations of acetylcholine or succinylcholine required to evoke, in the presence of the given concentrations of pachycurare drugs, muscle contractions equal to those evoked by acetylcholine alone at a concentration of 1×10^{-6} moles/l or succinylcholine alone at a concentration of 4×10^{-7} moles/l. On the basis of the data so obtained, we calculated the difference $pA_2 - pA_{10}$, using the equation of H. Schild [7]; in cases of competitive antagonism this difference equals log 9, or 0.95.

In the second series of experiments we studied the magnitude of contraction† of the rectus muscle (in percent of maximum) as a function of the concentration of acetylcholine or succinylcholine, in the presence and absence of constant concentrations of pachycurare drugs.

It is well known that the equation describing the reaction of a drug with a specific receptor can be derived on the basis of the law of mass action as applied to monomolecular reactions [3]. In this case the relation between the magnitude of contraction of the rectus muscle and the concentration of acetylcholine (or succinylcholine) is expressed by the equation

$$y = \frac{100[\mathrm{ACh}]}{K + [\mathrm{ACh}]} \qquad (I)$$

where y is the response to acetylcholine (succinylcholine) in percent of the maximal response, which is taken as 100; [ACh] is the concentration of acetylcholine (succinylcholine) in moles per liter; and K is the dissociation constant for the acetylcholine-receptor (succinylcholine-receptor) complex.

In the presence of a competitive antagonist that reacts with the same receptors, also in a monomolecular reaction, the dependence of the magnitude of muscle contraction on the acetylcholine (succinylcholine) concentration will be expressed by the equation

$$y = \frac{100[\mathrm{ACh}]}{K\left(1 + \frac{[A]}{K_A}\right) + [\mathrm{ACh}]}, \qquad (II)$$

*The designation "pachycurare" is applied to true curariform drugs, which inhibit membrane depolarization and are supposed to have a competitive type of action, as distinguished from "leptocurare," comprising those relaxants, such as succinylcholine, having a cholinomimetic action and presumably exerting their effect through depolarization. Paramyon is listed in Lekarstvennye Sredstva (Drugs) as meso-3,4-diphenylhexane-bis-p-trimethylammonium iodide, and Diplacine as 1,3-di(β-platynecinium ethoxy)benzol chloride [Translator's note].

†Presumably, the height of isotonic contraction was studied, but the Russian original is not specific [Translator's note].

where [A] is the concentration of antagonist in moles per liter, and K is the dissociation constant of the antagonist-receptor complex.‡

Equations (I) and (II) may be converted into straight-line equations having the general form $y = a + bx$, by transforming them according to the method of double reciprocals:

$$\frac{1}{y} = \frac{1}{100} + \frac{K}{100} \cdot \frac{1}{[ACh]}, \qquad (Ia)$$

$$\frac{1}{y} = \frac{1}{100} + \frac{K}{100}\left(1 + \frac{[A]}{K_A}\right)\frac{1}{[ACh]} \qquad (IIa)$$

If we compare Equations (Ia) and (IIa), we see that in a Lineweaver-Burk plot [6], i.e., in plotting reciprocals, the dependence of magnitude of contraction of the rectus muscle on the concentration of acetylcholine (succinylcholine) ought to be represented by a straight line cutting the ordinate at 0.01, both in the presence and in the absence of a competitive antagonist, but the slope of the line increases when a competitive antagonist is added, to a value of $(1 + [A]/K_A)$.

RESULTS

If we determine the values of pA_2 and pA_{10} in Fig. 1, we find that for the combination acetylcholine + tubocurarine $pA_2 - pA_{10} = 7.20 - 6.15 = 1.05$; for acetylcholine + Paramyon, $pA_2 - pA_{10} = 6.65 - 5.30 = 1.35$; for acetylcholine + Diplacine, $pA_2 - pA_{10} = 7.20 - 5.50 = 1.70$; for succinylcholine + Diplacine, $pA_2 - pA_{10} = 7.20 - 6.00 = 1.20$; and for succinylcholine + tubocurarine, $pA_2 - pA_{10} = 7.30 - 6.30 = 1.00$.

Examination of these calculated differences reveals that all of them are greater than 0.95, and consequently, the antagonism between pachycurare drugs and depolarizing agents is not strictly a competitive antagonism.

This conclusion is supported by the second group of experiments. As may be seen from Fig. 2, the curves representing the magnitude of contraction of the rectus muscle as a function of acetylcholine concentration, in the presence of tubocurarine at a concentration of 6×10^{-7} moles/l and Paramyon at 2×10^{-6} moles/l, conform completely to the theoretical concepts cited above as to the dependence of the magnitude of muscle contraction on the concentration of depolarizing agent in the presence of a competitive antagonist.

Thus, there is competitive antagonism between acetylcholine, on the one hand, and tubocurarine and Paramyon in these concentrations, on the other. But in the presence of tubocurarine in a concentration of 2×10^{-6} moles/l, or Paramyon in a concentration of 6×10^{-6} moles/l, the relation between the magnitude of contraction and acetylcholine concentration becomes nonlinear, and is expressed by curves approaching a parabola in form. A curvilinear relationship is also observed in the presence of Diplacine at a concentration of 6×10^{-6} moles/l.

Similar relationships are seen when pachycurare drugs are combined with succinylcholine: The reciprocal of the magnitude of muscle contraction is a linear function of the reciprocal of succinylcholine concentration, when the tubocurarine concentration is low, but as the tubocurarine concentration increases, this dependence becomes curvilinear (Fig. 3).

In addition, all the curves (see Figs. 2 and 3) intersect the ordinate at the value 0.01; it follows that the curvilinear relationship cannot be taken as proof that the antagonism between pachycurare drugs and depolarizing agents is not competitive in nature, since the slope and the intercept change in the presence of a noncompetitive antagonist.

The curvilinear relationship demonstrated in these experiments cannot be the result of a change in the value of K_A, since the affinity constants of quaternary ammonium bases, particularly tubocurarine, do not

‡Equations (I) and (II) are analogous to the equation of A. J. Clark [3] and J. H. Gaddum [4].

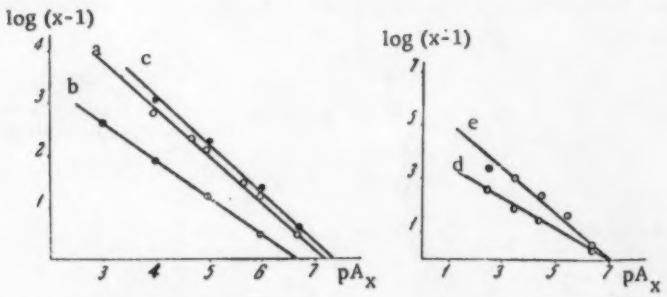

Fig. 1. The dependence of log (x–1) on the corresponding values of pA_x, for various pairs of drugs. a) Acetylcholine plus tubocurarine; b) acetylcholine plus Paramyon; c) succinylcholine plus tubocurarine; d) acetylcholine plus Diplacine; e) succinylcholine plus Diplacine.

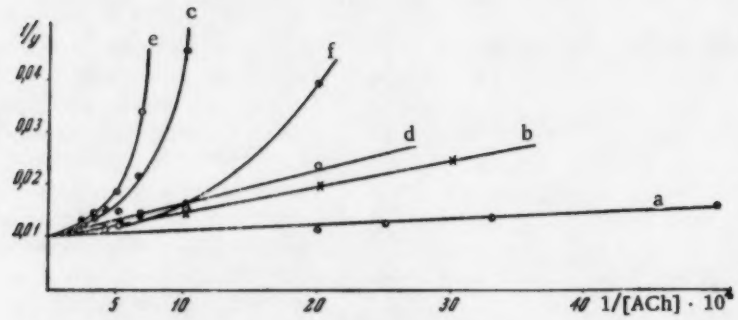

Fig. 2. Magnitude of rectus muscle contraction (on reciprocal scales) as a function of acetylcholine concentration, in the presence and absence of pachycurare drugs. a) Acetylcholine; b) acetylcholine plus tubocurarine (6×10^{-7} moles/l); c) acetylcholine plus tubocurarine (2×10^{-6} moles/l); d) acetylcholine plus Paramyon (2×10^{-6} moles/l); e) acetylcholine plus Paramyon (6×10^{-6} moles/l); f) acetylcholine plus Diplacine (6×10^{-6} moles/l).

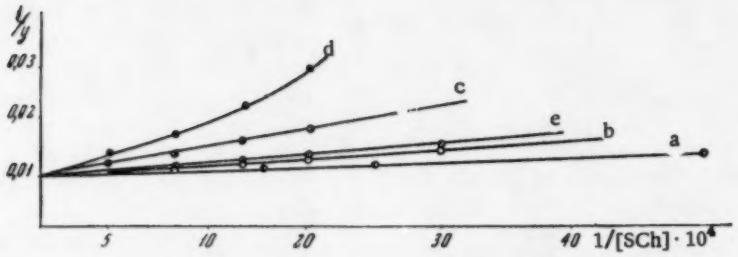

Fig. 3. Magnitude of muscle contraction (on reciprocal scales) as a function of succinylcholine concentration in the presence and absence of pachycurare drugs. a) Succinylcholine; b) succinylcholine plus Paramyon (2×10^{-6} moles/l); c) succinylcholine plus tubocurarine (2×10^{-6} moles/l); d) succinylcholine plus tubocurarine (4×10^{-6} moles/l); e) succinylcholine plus Diplacine (4×10^{-6} moles/l).

Certain Criteria Characterizing the Type of Antagonism Displayed by Pachycurare Drugs Toward Acetylcholine and Succinylcholine, Compared with Affinity Constants of Combined Substances

Combination	K_{aff} (antagonist-receptor)	K_{aff} (antagonist-receptor)	$pA_2 - pA_{10}$	Inhibition index at a pachycurare drug conc. of the order of		Reduction factor
				10^{-7}	10^{-4}	
Acetylcholine	$0,65 \cdot 10^6$	—	—	—	—	—
Succinylcholine	$1,22 \cdot 10^6$	—	—	—	—	—
Acetylcholine plus tubocurarine	—	$3,7 \cdot 10^6$	1,05	15	6,29	2,3
Acethylcholine+Paramyon	—	$1,64 \cdot 10^6$	1,35	3,8	0,84	4,5
Acethylcholine+Diplacine	—	$0,75 \cdot 10^6$	1,70	7,5	0,23	32,6
Succinylcholine + tubocurarine	—	$2,17 \cdot 10^6$	1,00	7,5	4,18	1,8
Succinylcholine + Diplacine	—	$0,33 \cdot 10^6$	1,20	3,63	0,85	4,2

change as their concentration increases [8]. Since acetylcholine and succinylcholine react with choline-reactive structures according to the model of a monomolecular reaction (see Figs. 2 and 3), the curvilinear relation observed between the magnitude of muscle contraction and concentration of depolarizing agent in the presence of high concentrations of pachycurare drugs is the result of a change in the monomolecular character of the reaction of pachycurare drugs with these choline-reactive structures. Certain histamine antagonists react with specific receptors in similar fashion [9]. In this case, Equation (II) must be given in a more general form:

$$y = \frac{100\,[\mathrm{ACh}]}{K\left(1 + \frac{[A]^n}{K_A}\right) + [\mathrm{ACh}]},$$

where n is the number of molecules of pachycurare drug associated with a single receptor.

For acetylcholine plus tubocurarine (2×10^{-6} moles/l), n = 0.968; for acetylcholine plus Paramyon (6×10^{-6} moles/l), n = 0.987; and for succinylcholine plus tubocurarine (4×10^{-6} moles/l), n = 0.875.

These values of n can be understood on the assumption that pachycurare drugs at high concentrations block receptors in choline-reactive structures not only by chemical reaction but also by adsorptive binding.

The possibility that a number of substances undergo both chemical and adsorptive reactions with protoplasmic cell structures can be regarded as established [2].

Since adsorption bonds are less stable than chemical bonds, smaller concentrations of depolarizing agents are required to overcome the antagonistic action of high concentrations of pachycurare drugs than would be expected if only chemical reactions (of the monomolecular reaction type) took place between pachycurare drugs and choline-reactive structures. This is confirmed by a calculation of the inhibition indices, whose values decrease as the concentration of pachycurare drug increases [1] (see table).

If pachycurare drugs react with choline-reactive structures by both chemical and adsorptive mechanisms, the affinity constants (K_{aff}) determined in these experiments are over-all constants reflecting the rate of chemical and adsorption reaction. We should therefore expect that the greater the affinity constants of pachycurare drugs and the depolarizing agents (and consequently, the greater the specific weight of the chemical reaction of these substances with receptors), the more the antagonism of pachycurare drugs toward the depolarizing agents will approach a true competitive antagonism.

Examination of the data in the table bears out this supposition completely.

The role of the adsorptive component in the reaction of pachycurare drugs with the choline-reactive structures becomes greater as the concentration of pachycurare drug increases. At low concentrations of pachycurare drug (of the order of 1×10^{-7}-1×10^{-6} moles/l), the adsorption mechanism is practically absent, and the antagonism between pachycurare (in these concentrations) and the depolarizing agents is a true competitive antagonism.

SUMMARY

The author studied the antagonism between pachycurare-type drugs (d-tubocurarine chloride, Paramyon, and Diplacine) and depolarizing agents (acetylcholine and succinylcholine) in experiments on the frog rectus abdominis muscle. It was shown that pA_2-$pA_{10} > 0.95$ for all the combinations studied, and therefore, the antagonism between pachycurare drugs and depolarizing substances is not strictly competitive. In studying the relationship between the magnitude of muscle contraction and the concentration of depolarizing substance, the author established that pachycurare drugs in concentrations of about 10^{-7}-10^{-6} moles/l act as a competitive antagonist of the depolarizing agents. As the concentration of pachycurare drugs increases, the competitive nature of the antagonism changes; this is due to a change in the monomolecular character of the interaction between pachycurare drugs and choline-reactive structures. This change may be caused by simultaneous chemical and adsorptive blocking of specific receptors.

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EFFECTS OF 2,4-DINITROPHENOL ON THE ELECTROCARDIOGRAM IN THE FROG

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 97-100, August, 1960
Original article received August 17, 1959

The development of electrocardiography was just getting under way when it began to appear that particular components of the electrocardiogram (EKG), especially the T wave, are dependent on the state of metabolic processes in the myocardium [11, 17]. Analysis of a large volume of experimental and clinical data accumulated prior to 1936 gave G. F. Lang [5] grounds for the opinion that the T wave is based on biochemical processes in the myocardium, and that the use of the electrocardiographic method showed great promise for the study of cardiac metabolism.

The progress made by modern biochemistry in the study of the energetics of muscle tissue has aroused interest in the metabolism of energy-rich (macroergic) phosphorus compounds in the myocardium, and has led to the publication of works dealing with studies of this metabolism involving the use of electrocardiography. For example, it has been found that a reduction of ATPase activity in hypocalcemia results in prolongation of electrical systole (the Q–T interval), the most characteristic sign of so-called energetic-dynamic cardiac insufficiency [16]. Enhancement of the breakdown of macroergic compounds under the influence of epinephrine, and diminution of this breakdown under the influence of acetylcholine, are combined primarily with changes in the T wave [9]. In experimental hypertension in rabbits, a disturbance of energetic processes in the myocardium is manifested in the EKG through a reduction in the height of the T wave and a displacement of the S–T interval [10].

In electrocardiographic investigations extensive use has been made of the method, widely employed in experimental medicine, of toxic depression of respiratory and glycolytic phosphorylation with so-called inhibitors. Under the influence of agents that poison glycolytic phosphorylation-sodium monoiodoacetate and sodium fluoride, the amplitude of the slow component of the ventricular complex (T wave) increases; then the T wave assumes a diphasic form, becoming less diphasic as the action of the poisons is prolonged; at the same time the initial portion of the EKG, the R wave, remains unchanged [3]. Methylene blue, an agent that causes dissociation of respiration and phosphorylation, leads in high concentrations to prolongation of the S–T interval and to a slight elevation of the T wave in the EKG of the frog [4].

We have been unable to find published reports of changes produced in the EKG of the frog by 2,4-dinitrophenol (DNP), the substance most widely used for the purpose of dissociating respiration from phosphorylation. The present paper is concerned with an investigation of this phenomenon.

METHOD

Experiments were carried out in the fall and winter months. The frogs (Rana temporaria) were immobilized with curare or urethan. The DNP solution was injected into the femoral lymph sac. Since the cessation of pulmonary respiration that occurs in animals under the influence of curare or urethan markedly enhances the toxic effect of DNP [6, 7], we injected DNP in doses (5-20 γ/g) that produced no visible changes in intact animals, but were lethal for immobilized animals. We were forced to abandon attempts to record the EKG with nonpolarizing electrodes, in spite of the fact that, as is well known from special studies [15], bringing the electrodes close to the heart of the frog results in a fuller representation of the true nature of the heart's electrical activity. After some orienting experiments with the use of direct recording, we chose to record the EKG in the standard leads, using steel needles implanted under the skin of the extremities and connected by wires to an ÉKP-4M electrocardiograph. The choice of standard leads was dictated mainly by two considerations: 1) to record changes in the electrical activity of the heart under the influence of sublethal doses of DNP from the moment of injection of the poison to the onset of cardiac arrest, one must record for 3-4 hours; but 1½-2 hours after the heart is exposed, the distortions in the EKG, owing to a variety of causes (drying, etc.), become considerable [11, 12, 13]; 2) in the course of these experiments it was found that the exposed heart stops

beating later than the nonexposed heart in DNP poisoning (this is evidently related to the diffusion of oxygen through the epicardium); under our experimental conditions this factor is not susceptible to precise quantitative evaluation, and so complicates the method. By comparing the three standard leads we established that the waves were more pronounced in lead III (left forelimb – left hindlimb); consequently, we thereafter recorded the EKG in lead III in all the frogs (40 experiments on 51 animals, of which 11 were controls). The over-all form of the EKG of curarized (or urethanized) frogs in our experiments was quite similar to those cited in published reports [8, 14].

RESULTS

The initial changes produced by DNP are slowing of the heart rate (before poisoning, 36-52 beats/min) shown by a lengthening of the R–R interval, and a marked elevation and deformation (diphasic form) of the T wave (Fig. 1). In the terminal period the form of the T wave returns to normal, or nearly so. As the toxic condition develops, the bradycardia progresses. Subsequently, there occurs a prolongation of the P–R interval (slowing of atrioventricular conduction) and the R–T interval (slowing of electrical systole), developing much more intensively than the slowing of the rate (before poisoning, the duration of these intervals was 0.35-0.42 sec and 0.65-0.92 sec, respectively). Thus, on comparison of frames 4 and 5 in Fig. 1, we see that, against the background of developing bradycardia, some acceleration of heart rate has occurred, and the duration of the R–T interval has increased. In those cases in which the S peak was distinctly evident before poisoning, it decreased in amplitude or disappeared as the poisoning progressed. The Q wave, in agreement with published data [1, 17], was not seen in the normal frog EKG in our experiments. All the EKG changes described were also seen in experiments with preliminary atropinization.

The almost constant invariability of the R wave observed in DNP poisoning of the heart provides a basis for the assumption that the process of depolarization, of which the R wave is the reflection, and the subsequent polarization of the heart, are relatively independent of the state of respiratory phosphorylation (an analogous assumption is made in regard to the glycolytic process). Just before cardiac arrest, the splitting of the ventricular complex described by E. I. Borisova and V. S. Rusinov[2]

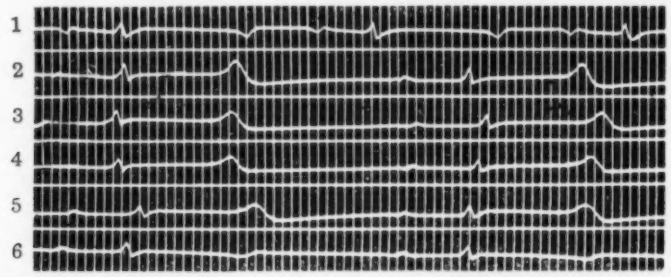

Fig. 1. Changes in the EKG of the frog under the influence of DNP. Time marker, 0.05 seconds. 1) Background (frog immobilized with curare, 2 γ/g; heart rate 35 beats/min); 2, 3, 4, 5, 6) 15, 47, 60, 90, and 120 minutes, respectively, after injection of DNP, 20 γ/g (heart rate 26, 24, 24, 28, and 28 beats/min, respectively).

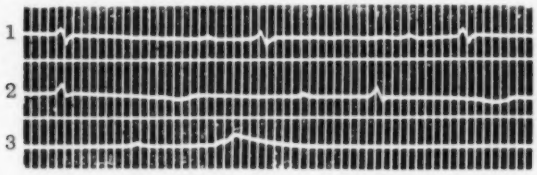

Fig. 2. Changes in the EKG of the frog under the influence of DNP. Time marker, 0.05 seconds. 1) Background (frog immobilized with urethan, 3 mg/g; heart rate 52 beats/min); 2, 3) 60 and 90 minutes, respectively after injection of DNP, 20 γ/g (heart rate 33 and 16 beats/min, respectively). Splitting of ventricular complex, of the type seen in longitudinal dissociation of single-chambered ventricle.

was observed, of the type seen in longitudinal dissociation of a single-chambered ventricle (3, Fig. 2).

These principal changes in the EKG produced by DNP gives evidence that when respiratory phosphorylation is depressed, the primary disturbances are disturbances in sinus automatism, in the restorative processes (of which the T wave is an expression) that ensure the spread of excitation, in atrioventricular conduction, and in electrical systole.

SUMMARY

The principal changes produced in the EKG of the frog by 2,4-dinitrophenol, an agent that dissociates respiration from phosphorylation, are (1) progressive slowing of the heart rate, (2) marked elevation and deformation of the T wave (it becomes diphasic), (3) lengthening of the P–R interval (slowing of atrioventricular conduction) and the R–T interval (slowing of electrical systole), and (4) diminution or disappearance of the S wave, developing much more rapidly than the slowing of the heart rate.

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THE EFFECTS OF GENERAL ANESTHETICS, ANALGESICS, AND CHLORPROMAZINE ON REFLEX REACTIONS ORIGINATING IN THE VESSELS OF THE PULMONARY CIRCULATION

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(Presented by Active Member AMN SSSR V. V. Zakusov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 100-105, August, 1960
Original article submitted August 14, 1959

It is well known that if veratrine [14], serum [15], or other substances are injected into the circulation, complex reflex reactions arise, in the form of hypotension, bradycardia, and changes in the rate and depth of respiration. It has been shown that these reactions are the result of afferent impulses from various vascular receptor zones: the coronary vessels [16, 17, 18, 24], the carotid sinus zone [21], and, especially, the pulmonary vessels. The last of these has been demonstrated by an original method involving the injection of stimulating substances (veratrine, aconitine, nicotine, and ammonium chloride) into a lobe of the lung that has been isolated from the circulation [5], and of veratridine, by catheterization of the right side of the heart or the pulmonary artery [13].

The latent period for reflexes originating in the receptors of the pulmonary vessels is 3-4 seconds, when the stimulating substance is injected into the right atrium [19]. The reflex reactions that appear after 8-10 seconds or more can be attributed to the influence of the chemical substances on the receptors of the coronary vessels, as well as their direct effect on the central nervous system [23].

Since afferent impulses from the receptors of the pulmonary vessels reach the brain stem only by way of fibers in the vagus nerve, the central components of these reflexes have synapses in the more caudal portion of the medulla. It is known from the work of V. V. Zakusov [6, 7, 8] that reflexes having synapses at this level in the central nervous system – e.g., the respiratory reflexes – are characterized by their stability to general anesthetics. This is in agreement with the fact that physiological investigations of reflexes from the lungs have usually been carried out on animals under general anesthesia. At the same time, individual general anesthetics show marked differences in their effects on different portions of the reticular formation of the brain stem [3, 4]. There is no doubt that depression of the functions of various brain stem structures by general anesthetics, analgesics, and neuroplegics can affect the character of reflexes from the pulmonary vessels.

The paucity of investigations in this area and the practical interest in the depression of reflexes from the lungs led us to study the effects of urethan, pentobarbital, hexobarbital, morphine, promedol, and chlorpromazine on the complex reflex reactions arising in response to injection of the stimulating agent into the vessels of the pulmonary circulation.

Injection of Stimulating Substances into a Pulmonary Lobe Isolated from the Circulation

Experiments were carried out by the method developed in the Institute of Pharmacology and Chemotherapy, USSR Academy of Medical Sciences, on cats anesthetized with urethan (1.3 mg/kg intraperitoneally). In this series of experiments we studied the effect of morphine and promedol on the hypotension and bradycardia originating reflexly from the receptors of the pulmonary vessels. Each experiment was performed on two cats, one of which served as donor, supplying blood to a lobe of the other cat's right lung, which was isolated from the rest of its circulation. In the second cat, femoral artery blood pressure and pulse were recorded on a kymograph drum by means of a mercury manometer. The donor underwent bilateral vagotomy for the prevention of reflex cardiovascular reactions on the donor side. Following data from special studies in this area [20], bovine serum stored for 8-10 weeks was used as a stimulating agent, and when 3-4 ml of this was injected into the artery of the isolated pulmonary lobe, typical reflex reactions resulted. Artificial respiration was used for both cats. Because of the desensitization of vascular receptors to serum [18], as well as to other substances [5], morphine and promedol were injected into the femoral vein in doses of 1 mg/kg ten minutes before serum was injected into the isolated lobe.

Hypotension and bradycardia were observed both after promedol and after morphine. In three experiments analgesics were injected during the period between the first and second serum injections. In this group of experiments morphine failed to reduce the hypotensive reaction, and the bradycardia was even more pronounced (Fig. 1). The change in the magnitude of reflex reactions under the influence of promedol was difficult to evaluate, since the observed diminution might result from desensitization of the receptors to the stimulus.

Thus, from these experiments we could conclude that both morphine and promedol are ineffective in preventing these reflex cardiovascular reactions from the receptors in the pulmonary vessels. The change in the magnitude of these reflex reactions under the influence of analgesics was investigated in the next series of experiments.

Injection of Stimulating Substances into the Right Atrium

Investigations were carried out on cats anesthetized with urethan injected intraperitoneally and supplementally by vein, and also on rabbits without anesthesia. Stimulating substances were injected into the right atrium by a catheter; in experiments on cats the substance used was serum, and in experiments on rabbits it was veratrine hydrochloride (15-20 μg in 0.005% solution), since serum evoked a barely perceptible reaction in rabbits. Respiration was recorded, and blood pressure and pulse were recorded from the common carotid artery. In estimating the magnitude of the hypotensive reaction in these experiments, we considered only the "primary" hypotension determined 5-6 seconds after injection of the stimulating substance into the right atrium and reflecting reflexes exclusively from the pulmonary vessels [19]. In view of the fact that a number of authors [5, 18, etc.], have reported desensitization of receptors of the pulmonary vessels upon frequent injection of stimulating substances, these substances were injected at intervals of 20-25 minutes. Under these conditions the reflex reactions retained their intensity through 4-5 repeated injections of serum into cats and veratrine into rabbits, in control experiments. All the pharmacological substances were injected intravenously by the drop method.

In this series of experiments morphine and promedol in doses of 1 mg/kg showed an inconstant effect on the cardiovascular component of the reflex reaction in cats. Thus, out of eight experiments with promedol, hypotension and bradycardia increased (by 10-20%) in three (Fig. 2a), were briefly diminished (by not more than 30%) in four, and remained unchanged in one. Of five experiments with morphine, an increase (20%) in the hypotension and bradycardia was noted in three experiments, and no change was observed in two.

In the experiments on rabbits, injection of promedol in a dose of 1 mg/kg generally caused an initial reduction (40-50%) in cardiovascular reactions, with subsequent elevation to the original level. Morphine (in a dose of 1 mg/kg) had essentially no effect on the hypotension and bradycardia, or slightly enhanced them.

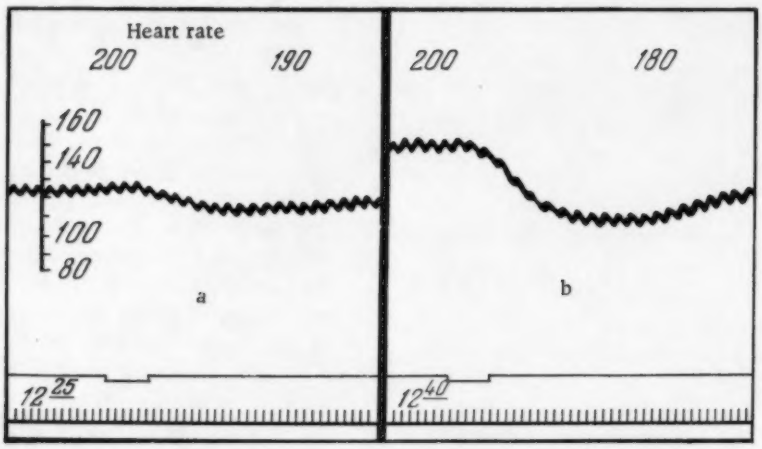

Fig. 1. Reflex reactions upon injection of serum (4 ml) into lobe of right lung isolated from circulation, before (a) and 13 minutes after (b) injection of morphine (1 mg/kg). Cat weighing 3.2 kg; anesthesia–urethan, 1.3 mg/kg intraperitoneally. Figures are heart rate calculated as beats per minute. Interpretation of curves (top to bottom): blood pressure, marker indicating serum injection, time marker (1 second).

As regards the respiratory component of this reaction– under the influence of analgesics, especially promedol, the appearance of an expiratory respiratory pause or the lengthening of this pause was observed. This pause was more pronounced in cats, particularly in those experiments where intensification of bradycardia was observed (see Fig. 2a). In rabbits this effect was observed later than in cats, and also coincided with the moment of intensification of bradycardia.

In the experiments on cats urethan in a dose of 1-1.25 mg/kg had no noticeable effect on the cardiovascular reactions. The character of the respiratory reflex was determined from the depth of anesthesia: in incomplete anesthesia an increase in the amplitude of respiration was observed, and when anesthesia was deepened by injection of urethan into the vein, a respiratory pause appeared in the expiratory phase (Fig. 2b). In rabbits hexobarbital in a dose of 35 mg/kg (2/3 of the anesthetic dose) completely reversed the bradycardia and reduced hypotension (by 40-50%). Pentobarbital in doses of 5 mg/kg in rabbits (1/5 of the anesthetic dose) and 10-15 mg/kg in cats (1/3-1/2 of the anesthetic dose) greatly weakened the whole reflex complex: the hypotension was depressed by 70-80%, and the bradycardia completely, the respiratory reaction also being reduced (Fig. 3).

In experiments on cats, chlorpromazine in a dose of 1-2 mg/kg reduced all three components of the re-

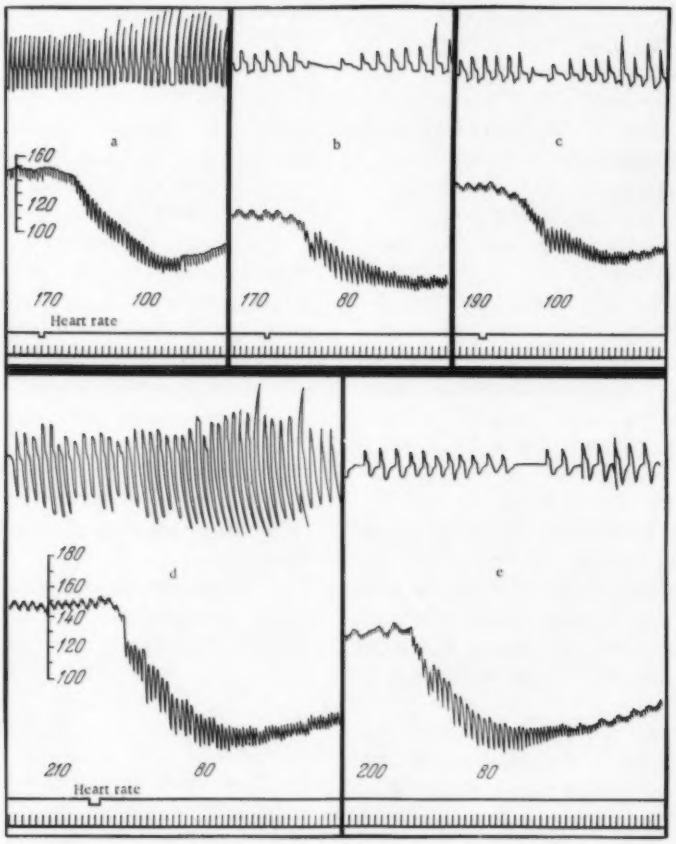

Fig. 2. I. Reflex reactions to injection of serum (0.8 ml) into the right atrium, before (a), ten minutes after (b), and 30 minutes after (c), injection of promedol (1 mg/kg). Cat (weight 4.8 kg); incomplete anesthesia – 1 mg/kg intraperitoneally. II. Reflex reactions to injection of serum (0.8 ml) into the right atrium in incomplete urethan anesthesia (d) and after anesthesia was deepened to become complete (e). Cat (weight 3.3 kg); anesthesia – urethan, 1 mg/kg intraperitoneally plus 0.3 mg/kg intravenously. Figures are heart rate calculated as beats per minute. Interpretation of curves (top to bottom): respiration (inspiration is downward), blood pressure, marker indicating stimulus injection into the right atrium, time marker (1 second). Designations as in Fig. 1.

flex reaction by roughly 50%. In this case bradycardia returned to its initial level after 1-1½ hours, whereas the hypotensive reaction remained at the reduced level. In experiments on rabbits, chlorpromazine in a dose of 1-1.6 mg/kg completely suppressed the whole reflex complex, and restoration of these reactions began two hours after it was injected. Since chlorpromazine lowered blood pressure by 40-50%, we carried out control experiments on cats and rabbits, in which the blood pressure was lowered to the same extent by hemorrhage (20-25 ml of blood). The intensity of the hypotension was maintained to the same extent, or was reduced by not more than 10%, and the bradycardia was actually intensified.

It follows from the data above that reflex reactions originating in the receptors of the pulmonary vessels, with afferent impulses proceeding along the vagi, are affected to different degrees by different anesthetics. Urethan, as general anesthesia deepens, "facilitates" expiration without having any material effect on the hypotension and bradycardia. The change in the character of the respiratory reflex indicates that under the influence of this anesthetic the expiratory "center" in the cranial portion of the dorsolateral formation of the medulla is liberated from inhibitory influences exerted by higher divisions of the central nervous system. Thus, the depressant effect of urethan in the brain stem is slight, and in the usual anesthetic doses it does not reach the caudal portion. Pentobarbital in doses of 1/5-1/2 the anesthetic dose markedly inhibits the whole reflex complex; this shows that pentobarbital simultaneously depresses different brain stem structures at the same time, including those that are located in the caudal portion of the brain stem. With hexobarbital this effect is weaker than with pentobarbital. The diminution of bradycardia under the influence of barbiturates is explained, in addition, by their ganglion-blocking action [11, 12].

Morphine and promedol also have different effects on reflexes from the receptors of the pulmonary vessels, a species difference being noted in the action of the latter: in rabbits promedol first inhibits the entire reflex complex to some extent, and then "facilitates" expiration and enhances bradycardia; in cats, on the other hand, the first phase of the action of promedol is almost absent. In both rabbits and cats morphine either has no noticeable effect or else enhances hypotension and bradycardia and "facilitates" expiration. A difference in the action of these two analgesics has also been observed in relation to other effects. For example, promedol is more active than morphine in depressing reflexes originating upon stimulation of the deep portions of the respiratory tract [9], and in depressing inhibition of the knee-jerk reflex elicited by electrical stimulation of the cerebellum [1]. Apparently the depressant effect of promedol in the central nervous system is less selective than is the case with morphine.

Chlorpromazine inhibits the whole reflex complex originating in the pulmonary vessels to a greater or lesser extent, depending on the species of the animal, which is in agreement with data on chlorpromazine suppression of most of the inhibitory and facilitatory structures in the brain stem [10].

The depressant action of chlorpromazine, promedol, and pentobarbital on reflexes from the pulmonary vessels is more constant and more marked in rabbits than in cats. This species difference probably is connected with the functional predominance of the descending activating systems in cats as compared with these systems in rabbits. This may also explain certain peculiarities in the character of the reflexes. For example, in cats the amplitude of respiration increases as a consequence of the intensification of expiration, but in rabbits, inspiration is intensified; in cats, too, bradycardia and hypotension are usually more pronounced. The same species peculiarities in these reflexes have also been observed following injection of serotonin into the circulation [22].

The "facilitation" of expiration and bradycardia observed under the influence of urethan and analgesics should be regarded as the result of the removal by these substances of inhibitory influences from other portions of the central nervous system [2, 3, 4].

Of the analgesics, promedol exerts a diphasic effect on reflexes from the pulmonary vessels in the rabbits, first inhibiting them, and then enhancing bradycardia and "facilitating" expiration. In cats the first phase in the action of promedol is slight and irregular. Morphine either has no effect on these reflexes or else enhances them.

Chlorpromazine completely suppresses the entire reflex complex in rabbits, and in cats, it depresses the complex by not more than 50%.

SUMMARY

The results of the experiments described in this paper demonstrate that the reflex reactions to injection of a stimulating substance into the pulmonary circulation change differently under the influence of different anesthetics: Urethan "facilitates" expiration without affecting the cardiovascular component; pentobarbital tends to depress the whole reflex complex to various degrees; while hexobarbital primarily depresses the bradycardia. The bradycardia may also be reduced on account of the ganglionic blocking action of the barbiturates. Promedol, an analgesic, has a diphasic effect on the reflexes from the pulmonary vessels: It first decreases them and then intensifies bradycardia and "facilitates" expiration. In cats the first phase of the promedol effect is slight and irregular. Chlorpromazine depresses the entire reflex complex completely in rabbits, but by not more than 50% in cats.

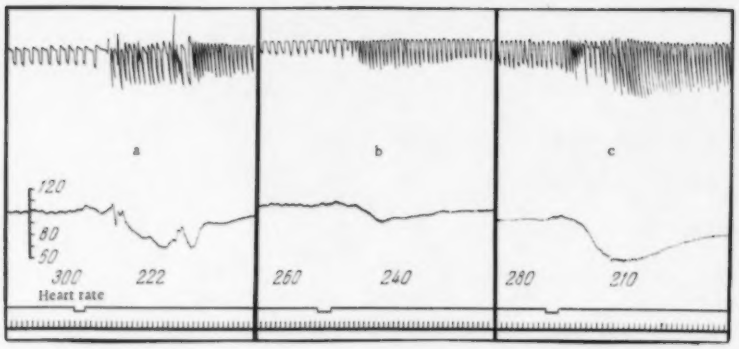

Fig. 3. Reflex reactions upon injection of veratrine hydrochloride (20 μg) into the right atrium, before (a), 5 minutes after (b), and 25 minutes after(c), injection of nembutal (5 mg/kg). Rabbit (weight 2.6 kg); without anesthesia. Designations as in Fig. 2, I.

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DETECTION OF CENTERS OF SUCCINODEHYDROGENASE ACTIVITY IN Escherichia coli

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 106-107, August, 1960

Original article submitted October 3, 1959

The survival of bacteria after freezing and drying is a problem of great importance in the preparation of live vaccines. The explanation of the causes of disturbance of the vital activity of the cell during intensive cooling and freeze-drying is of great theoretical and practical interest. The disturbance of vital activity during deep cooling may be connected with the denaturation of proteins, especially enzyme proteins, and with changes in the kinetics of metabolic processes during alterations in the temperature [5].

There are reports in the literature of the lability of certain enzymes connected with the mitochondrial fraction of animal tissues at a low temperature, among them certain dehydrogenases, notably succinodehydrogenase [1, 3, 6, 7, 8].

Since succinodehydrogenase is an important component of the final stage of cell respiration, any degree of denaturation of the protein of this enzyme may result in considerable changes in the vital activity of the cell. It is therefore important to investigate the changes in this enzyme in the bacterial cell when subjected to the action of low temperatures and dehydration.

EXPERIMENTAL METHOD

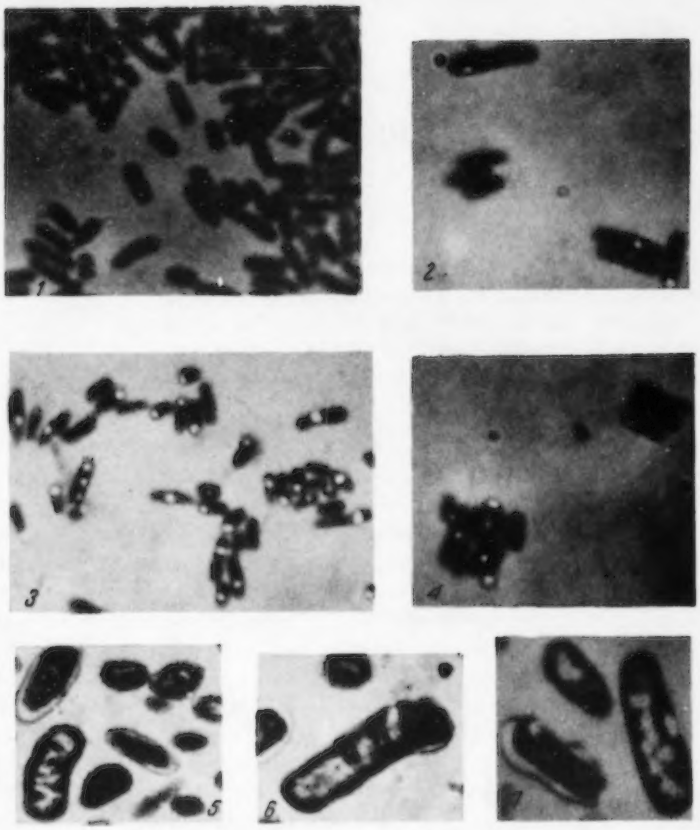
For the investigation, Escherichia coli strain 894 was used, which has typical morphological and cultural properties. Incubation was for 17.5 hr on meat-peptone agar at 37° with 2, 3, 5-triphenyltetrazolium chloride in the presence of sodium succinate in various concentrations and Ca^{++} ions (10^{-5} M) or cystine (M/2000-M/8000) as enzyme activators, or of sodium arsenite in various concentrations as inhibitor. Observations were made after different periods of incubation (from 10 to 60 minutes). Microscopy by means of a phase-contrast apparatus; objective FI90 ×, ocular 10 ×. Photography in the same conditions on a "Mikrat" film; exposure 8 seconds; photographic adaptor MFN-1 with MFK-3 camera. Focusing was carried out on formozan crystals, so that the cell contours were somewhat blurred. Part of the material was treated for preparation of ultrafine sections. Electron microscopy: microscope – ÉM-3, at 50 kv; magnification 5700-7300. The formozan was extracted by passage through alcohol, so that spaces where the crystals must have been are seen in the sections, and not the crystals themselves. The experimental material was investigated quantitatively with respect to formozan formation by the method of Fahmy and Walsh [2].

EXPERIMENTAL RESULTS

The specificity of the reaction was demonstrated. In the absence of sodium succinate in the washed E. coli suspension, no reduction of tetrazolium was observed after incubation for 60-120 minutes (see figure). In no case of work with a well-washed culture did we find formozan granules outside the cells.

After the addition of sodium succinate in different concentrations, the formation of formozan was observed (see figure, 2, 3). The higher the concentration of succinate, the more intensively was formozan formed. Ca^{++} ions and cystine activated the reaction. Arsenite depressed it significantly (see figure, 4). In the cells 1-2 formozan granules were most often found, distributed as previously described by Niklowitz [4], at both ends, or at one end and in the middle, close to the cell wall (see figure, 2). In one case we observed three formozan granules in the cell (see figure, 3). The grains were situated close to the cell wall (this is clearly seen in the early period of reduction, or after incubation with small concentrations of succinate).

In ultrathin sections of the bacteria the same localization is observed (by the wall; see figure, 5-7). The sites of reduction apparently border the cell membrane, being in the thickness of the cytoplasm. It cannot be concluded from our electron photomicrographs that the sites of reduction of tetrazolium differ in any way from



Reduction of triphenyltetrazolium chloride by Escherichia coli. 1) Control (E. coli 894 + tetrazolium chloride); 2) tetrazolium chloride + 0.005 M sodium succinate; 3) tetrazolium chloride + 0.05 M sodium succinate; 4) tetrazolium chloride + 0.05 M sodium succinate + $M/10^4$ sodium arsenite; 5) control (ultrathin sections); 6 and 7) incubation with 0.1% tetrazolium chloride.

the rest of the cytoplasm. Niklowitz [4] found that the respective areas could be detected morphologically in the cell before the reaction with tetrazolium.

The strict localization of the sites of succinodehydrogenase activity provides evidence of a definite morphological substrate of action of the enzyme. Since succinodehydrogenase activity is associated in tissues with the mitochondrial fraction, we may speak of such centers in the bacterial cell as functional equivalents of the mitochondria.

I would like to express my gratitude to N. N. Solov'ev for his great assistance in this work.

SUMMARY

2, 3, 5-Triphenyltetrazolium chloride is a specific reagent for succinodehydrogenase in the Escherichia coli cell. A cell is found to have from 1 to 3 "activity centers" of succinodehydrogenase. Since the tissue succinodehydrogenase is associated with mitochondria, one may speak of mitochondria equivalents in the bacterial cell.

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RELATIONSHIP BETWEEN ADSORPTION OF IMMUNE ANTIBODIES AND THE CHEMICAL STRUCTURE OF THE ADSORBENTS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 107-111, August, 1960
Original article submitted October 20, 1959

Adsorption methods, widely used in various branches of chemistry, are now beginning to be used in immunological research. The laws governing the selective adsorption of the proteins of immune sera are still, however, inadequately studied. The work of Landsteiner and Stankovic [8], Eisler and Tsuru [6], Friedberger and Putter [7], Bleyer [2], Eisler [3, 4], Eisler and Spiegel-Adolf [5], V. N. Shreder [1], and Olitzki [9] has shown that typhoid and dysentery agglutinins, diphtheria and tetanus antitoxins from immune sera, and also hemagglutinins are adsorbed by activated charcoal, kaolin, kieselguhr, white clay, aluminum hydroxide, ferric hydroxide, and collargol. The adsorption of antibodies by these adsorbents was not selective, and other proteins were adsorbed along with the agglutinins from the immune sera. In Bleyer's [2] experiments, for instance, after adsorption of a typhoid immune serum with charcoal, no agglutinins could be found in it, and tests for proteins with sulfosalicylic acid were negative. According to V. N. Shreder's findings [1], 2 g of freshly prepared aluminum hydroxide completely adsorbed the hemagglutinins from 20 ml of serum, and 91-100% of the other proteins. We found no information on the relationship between the adsorption of normal and immune antibodies in the sera and the chemical structure of the adsorbents in the literature accessible to us.

In the present paper we describe the results of our investigations into the laws governing the adsorption of agglutinins by different adsorbents.

EXPERIMENTAL METHOD AND RESULTS

In the first place we investigated the relationship between the adsorption of antibodies and the chemical structure of the adsorbents. For this purpose we tested the agglutinin-adsorbing power of substances of differing chemical structure, only slightly soluble in water. The following compounds were studied: 1) those containing amino groups: benzidine, α-naphthylamine, thionine, and the anion exchange resins ÉDÉ 10 and ÉDÉ 10P; 2) those incorporating nitro groups: mononitronaphthalene trinitrotoluene, and 2,4-dinitrodiphenylamine; 3) those containing hydroxyl groups: β-naphthol, 8-hydroxyquinoline, phenol red, rosolic acid; 4) those containing quinone groups: naphthaquinone, anthraquinone; 5) those containing aldehyde groups: p-dimethylamidobenzaldehyde; 6) those containing carboxyl groups: naphthalenetetracarbonic acid, methyl orange; 7) those containing sulfo groups: naphthalene-1,6-disulfonic acid and the cation exchange resins KU-1 and KU-2; 8) those containing chlorine: p-dichlorobenzene, polyvinyl chloride, DDT, hexachlorocyclohexane; 9) organic compounds containing metals: dermatol, bismuth salicylate, bismuth citrate, carmine; 10) compounds not containing functional groups: naphthalene, acridine, anthracene; 11) compounds containing certain functional groups: salicylic acid, anthranilic acid, thymol blue, tyrosine, alizarin, α-aminoanthraquinone, α-aminohydroxyanthraquinone, 2,4-dinitrochlorobenzene, m-nitroaniline and 2,4-dinitrophenylhydrazine.

The experimental method was as follows: To 2 ml of crude agglutinating rabbits' sera, diluted 1:100 in physiological saline (pH = 7.2) was added 100 mg of the test substance in powder form, after it had first been sifted through a standard brass mesh of gauge 40μ. The sera with the adsorbents were kept at a temperature of 20° for 20 minutes and periodically stirred with a glass rod. After an exposure of 20 minutes, the mixture of adsorbent and serum was centrifuged to complete translucency, and the naked-eye agglutination test carried out with the supernatant serum in tubes by the usual method with the aid of formol diagnosticums. For control purposes, naked-eye agglutination tests were carried out with sera which had not been adsorbed with test substances. The results of the tests were read by means of a loupe. The difference between the titer of agglutinins in the unadsorbed serum and in the serum adsorbed with the test serum showed the adsorptive activity of this substance. When evaluating the results of adsorption of agglutinins, we took into consideration the fact that fine powders are capable of absorbing small quantities of protein irrespective of their chemical structure. If, therefore, after adsorption of an immune serum with a chemical substance, the intensity of agglutination was

TABLE 1. Relationship Between Adsorption of Typhoid H-Agglutinins and Chemical Structure of Adsorbents

Organic compounds	Functional groups	Agglutinin titers after adsorption					Diagnosticum control
		1:10	1:20	1:40	1:80	1:160	
β-naphthol, phenol red	Hydroxyl	++++	++	±	−	−	−
Naphthalene-1,6-disulfonic acid	Sulfo groups	++++	+++	−	−	−	−
Cation exchange resin KU-2		+++	±	−	−	−	−
Naphthalenetetracarbonic acid	Carboxyl	++++	+++	±	−	−	−
p-Dimethylamidobenzaldehyde	Aldehyde	++++	+++	+	−	−	−
Naphthoquinone	Quinone	++++	+++	−	−	−	−
Anthraquinone	"	++++	+++	−	−	−	−
Alizarin	Quinone and hydroxyl	++	+	−	−	−	−
α-aminoanthraquinone	Quinone and amino	++++	++++	+	−	−	−
α-aminohydroxyanthraquinone	Quinone, amino and hydroxyl	++++	+++	−	−	−	−
Mononitronaphthalene	Nitro groups	++++	+++	+	−	−	−
Trinitrotoluene		++++	+++	+	−	−	−
α-naphthylamine	Amino groups	++++	+++	++	±	−	−
Thionine		++++	+++	++	±	−	−
Anion exchange resin ÉDÉ 10P		++++	+++	++	±	−	−
Hexachlorocyclohexane	Chlorine	++++	++++	++	±	−	−
Naphthalene		++++	++++	++	±	−	−
Anthracene		++++	++++	++	±	−	−
Control serum (unadsorbed)		++++	++++	++	+	−	−

Note. In diluting the sera, both control and adsorbed, the initial dilutions (1:100) were taken as unity. Serum controls (dilution 1:100) were negative.

TABLE 2. Intensity of Adsorption of Agglutinins by Certain Substances from a "Flexner C" Rabbit Agglutinating Serum

Adsorbents	Weighed samples of adsorbent in mg	Serum agglutinin titers after adsorption					Weight of protein adsorbed in mg	Protein adsorbed as % of total serum protein
		1:32	1:64	1:1:8	1:256	1:512		
Alizarin	500	++++	++	–	–	–	8.30	24.48
β-naphthol	The same	++++	++++	++	–	–	7.03	20.65
Naphthalene-1,6-disulfonic acid	"	++++	++	–	–	–	6.10	17.997
Anion exchange resin ÉDÉ 10P	"	++++	++++	+++	+	–	11.4	33.62
Agglutinin titer of unadsorbed serum (control)	"	++++	++++	+++	++	–	–	–

Note. Adsorption was carried out on 2.5 ml of serum, diluted 1:5 with physiological saline; + designates the intensity of agglutination; – absence of agglutination. In the dilution of the control and adsorbed sera, the initial dilutions (1:5) were taken as unity. Controls of sera and diagnosticum negative.

reduced only by +, this adsorption was considered to be nonspecific, i.e., independent of the chemical structure of the substance. If, on the other hand, after adsorption of the serum the agglutinin titer fell by one dilution or more, then in this case the adsorption was regarded as specific for the particular chemical substance (the titer of a serum was taken to be agglutination with an intensity of + +).

Investigation of the adsorptive power of the substances listed showed that organic compounds in whose molecules there are no functional groups (naphthalene, anthracene), did not adsorb agglutinins. Similarly, substances in whose molecules there are functional chlorine and amino groups did not adsorb agglutinins. Substances containing hydroxyl, aldehyde, carboxyl, quinone, sulfo, and nitro groups possessed the property of adsorbing agglutinins (compounds listed in the second, third, fourth, fifth, sixth, seventh, ninth, and eleventh groups). The magnitude of adsorption of agglutinins from typhoid agglutinating sera by some organic compounds is shown in Table 1.

It will be seen from the data in Table 1 that the intensity of adsorption of agglutinins by the majority of the tested substances was insignificant, and the titer of the agglutinating serum after adsorption by these substances fell by one or two dilutions. After adsorption of the agglutinating sera by substances not containing functional groups (anthracene, naphthalene) and also by substances having chlorine and amino groups in their composition, the intensity of agglutination fell by only +. Organic compounds containing nitro, aldehyde, and carboxyl groups adsorbed agglutinins only insignificantly. After adsorption of agglutinating sera by these substances the intensity of agglutination fell by + +. Of all the substances which we investigated, those which adsorbed agglutinins most intensively were compounds containing several hydroxyl groups – alizarin and phenol red. The latter completely adsorbed the agglutinins, and after adsorption of the immune sera with alizarin the agglutinin titer fell by two dilutions.

Comparison of the adsorptive activity of anthraquinone, α-aminoanthraquinone, and hydroxyaminoanthraquinone revealed the reciprocal influence of different groups on the intensity of adsorption of agglutinins. For instance, in a serum adsorbed by anthraquinone, the agglutinin titer fell by one dilution; after adsorption by α-aminoanthraquinone only the intensity of agglutination was reduced, but in a serum adsorbed with hydroxyaminoanthraquinone the agglutinin titer was lowered by one dilution. The introduction of an amino group into the anthraquinone ring thus weakens its adsorptive activity, but the introduction of hydroxyl groups considerably increases the adsorption of agglutinins by this compound.

In order to determine the extent to which the adsorption of agglutinins by organic compounds is selective, we carried out a comparative estimation of the fall in the agglutinin titer and the protein content of im-

mune sera after adsorption with certain organic compounds. Adsorption of the agglutinins was carried out by the method described above, but the sera used were diluted 1:5 with physiological saline; weighed samples of the adsorbents contained 500 mg. The protein content of the sera was determined by Kjeldahl's micromethod. The results of these experiments are given in Table 2.

We see that alizarin adsorbs 8.3 mg of protein and naphthalene-1,6-disulfonic acid, 7.03 mg of protein, but the agglutinin titers under these circumstances fell by only one or two dilutions. Consequently, the adsorption of agglutinins was not strictly selective, and nonimmune serum proteins were adsorbed, besides agglutinins, onto these particular substances. During the adsorption of the immune serum with anion exchange resin ÉDÉ 10P, containing amino groups, despite the considerable adsorption of serum protein, the agglutinins were not adsorbed.

During the investigation of the effect of certain conditions on the adsorption of agglutinins from immune sera by alizarin, it was found that changes in the pH of the medium within the limits of 6.0-7.4, and in the temperature between 6° and 37°, had no effect on the intensity of adsorption of agglutinins. Similarly, no difference was observed in the degree of adsorption of O and H agglutinins.

Experiments on the desorption of the agglutinins adsorbed on alizarin and cationite KU-2 showed that, in the process of adsorption, the agglutinins are firmly bound to the surface of the adsorbents.

Agglutinins adsorbed on the adsorbents listed above were not desorbed after prolonged agitation in dilute solutions of acids or alkalies, in physiological saline or in hypertonic saline solution.

SUMMARY

Adsorption of immune serum agglutinins by organic compounds was investigated. Compounds with hydroxyl, aldehyde, carboxyl, and quinone groups, as well as sulfo and nitro groups, were capable of adsorbing these agglutinins. Organic substances containing no functional groups, and substances devoid of chlorine and amino-groups did not adsorb agglutinins. Being firmly bound to the surface of adsorbents, the adsorbed antibodies are not desorbed by weak acid or alkaline solutions or by physiological or hypertonic saline.

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INVESTIGATION OF THE CELLULAR COMPOSITION OF THE BONE MARROW IN MONKEYS AFTER REPEATED PUNCTURE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 112-115, August, 1960
Original article submitted September 14, 1959

The study of the dynamics of the morphological composition of the peripheral blood in monkeys [1] has shown changes in the number of leukocytes per mm^3 after the repeated taking of blood samples, all the experimental conditions being the same. The high leukocyte count figures observed during the first days of the observations were appreciably diminished during the systematic repetition of the procedure of taking blood from the same animal under the same conditions. The fact that a depression of the leukocyte count takes place in monkeys under the influence of repeated blood investigations must be taken into consideration, not only when the physiological normal values for these animals are being established, but also when systematic investigations are being made for the purpose of studying the action of various agents on the blood.

We considered that it would be of interest to examine, in the same way, the dynamics of the cellular composition of the bone marrow in monkeys. In the conduct of our investigation we discovered facts which, although not connected with this depression phenomenon, were nevertheless of essential importance from the point of view of technique.

EXPERIMENTAL METHOD

The work was carried out on five male monkeys of the species Papio hamadryas, aged from one to two years. The animals were clinically healthy and were kept in isolation from the herd.

The site chosen for puncture of the bone marrow was the tibial tuberosity in both lower limbs.

During puncture the monkey sat in the attendant's arms. The leg was fixed vertically, supported on the foot; for convenience in finding the point of puncture the mobile skin surface of the animal's limb was drawn in the direction of the thigh. The skin at the site of puncture was not anesthetized, but after removal of the hair it was painted with alcohol.

Puncture in monkeys presents no special difficulty, for the site of puncture is readily determined. This point is situated at a distance of approximately 5-10 mm from the articular surface of the tibia.

The site of puncture was palpated with the thumb of the left hand, and with a rotary movement of the right hand a Kassirskii's needle was inserted. The moment of entry of the needle into the medullary cavity was determined by means of the stilet: if, on extraction of the stilet, traces of marrow contents could be seen upon it, it could be considered that the needle was situated within the medullary cavity. After extraction of the stilet, a syringe was attached to the end of the needle for aspiration of the marrow; the contents of the syringe were expelled onto a glass slide, whereupon five films were prepared.

In order to determine the absolute number of leukocytes in the bone marrow, the puncture specimen was collected in a red cell mixing chamber with acetic acid. In the investigation of the myelogram, 500 cells were counted in three films. Furthermore, samples of blood for analysis were taken at the same time from the right ear, and the total and differential leukocyte counts were determined. The experiments were carried out once a week, always during the morning hours before the animals had been fed.

The observations were continued for 6-9 months by the same person, and the experimental conditions remained constant throughout this time.

EXPERIMENTAL RESULTS

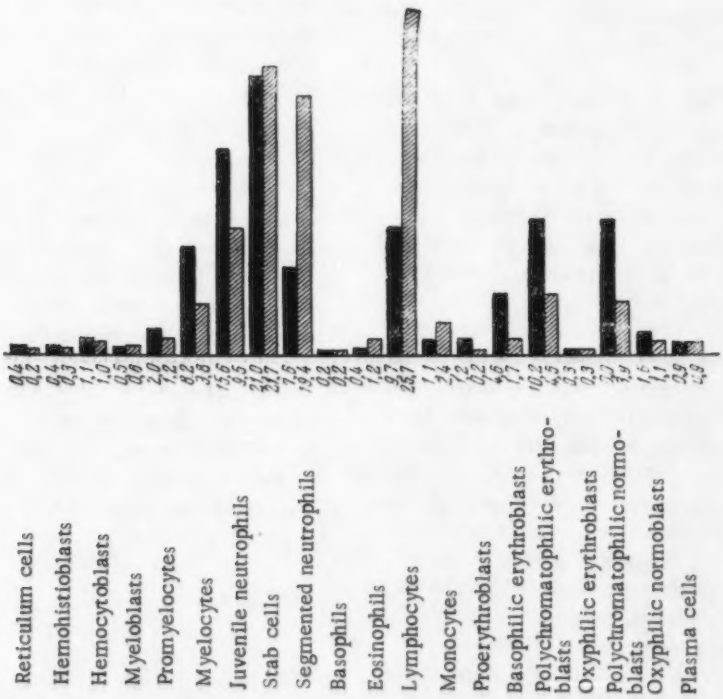
Repeated puncture of the same area of the marrow tissue leads to definite disturbances of the processes of hemopoiesis at that point. These disturbances were shown, in the first place, by a decrease in the number of leukocytes in this particular area of the marrow and by changes in the relative proportions of the cells obtained by puncture.

In the table are given the results (obtained in three monkeys: Koster, Vik, and Anyb), showing the absolute number of leukocytes in the marrow specimen as a result of the first and last experiments, and also the

Dynamics of the Absolute Number of Leukocytes in the Bone Marrow after Repeated Puncture

Name of monkey	Total number of punctures	Duration of experiments (in months)	Absolute number of leukocytes in the bone marrow				P (probability of random variation between mean indices obtained at beginning and end of experiment)
			first day of investigation	mean value in first four investigations	last day of investigation	mean value in last four investigations	
Koster	31	9.0	353 000	229 000 ± 41 000	22 000	27 000 ± 2 700	0.006
Vik.	24	5.5	137 000	229 000 ± 28 000	21 500	35 000 ± 4 000	0.001
Anyb.	24	5.5	324 000	238 000 ± 44 200	23 500	41 000 ± 9 700	0.011

number and duration of the investigations carried out. From the figures given, it follows that the absolute leukocyte count in the marrow specimen from the monkeys on the first day of aspiration of the marrow was very high, varying between 353,000 and 137,000. The mean number of leukocytes in the specimens obtained in the first four investigations was in no case less than 229,000. Repeated puncture of the same area of hemopoietic tissue led to an obvious decrease in the number of leukocytes in this area of the marrow, which varied between limits of 23,500 and 21,500. Statistical treatment of the results showed that the difference between the mean indices obtained at the beginning and end of the investigation was significant. The fall in the number of cells in this area of the bone marrow persisted for 3-4 months, and it was not restored in spite of an interruption of the work for 1½ months. In two monkeys (Antil and Isuden) a fall in the absolute number of leukocytes in the bone marrow appeared in the second or third week of puncture; in the first week of



Myelograms (in per cent) when the absolute number of leukocytes in the marrow was maximum and minimum. Black columns – proportion of cells of marrow specimen with maximum values; shaded columns – the same, with minimum values.

puncture its value was 329,500-157,000, and in subsequent weeks it fell to 24,000-16,000. In this period normal marrow activity was preserved in other areas of hemopoiesis (humerus, ilium) in all the monkeys. Whereas an average of 21,000 leukocytes (3 monkeys) was found in the tibia which was repeatedly punctured, the first puncture of the humerus, carried out at the same time, revealed 194,500 leukocytes, and puncture of the ilium – 105,000 leukocytes. It must be pointed out that normally the absolute number of leukocytes and the quantitative proportions of the cells in the bone marrow in the paired limbs are identical.

Analysis of the changes in the cellular composition of the bone marrow in the experiments described above revealed that this was directly connected with the changes in the leukocyte count. The figure illustrates the mean data of the myelograms (calculated in %) obtained when the absolute number of leukocytes in the bone marrow was maximal (from 350,000 to 100,000) and minimal (from 85,000 to 30,000).*

It follows from the data shown that in the first weeks of puncture, when the bone marrow is active and rich in cells, the relative proportions of the morphological indices are within the normal limits characteristic of this lower species of monkey. The percentage content of stab cells (21%) in the myelogram is higher than that of segmented neutrophils (7.6%), which is evidence of the rapid expulsion of mature cells into the blood stream.

The normal activity of the cells of the leukoblastic and erythroblastic series in this period is also shown by the following indices: the leuko-erythroblastic ratio was 2.4; the maturation index of the neutrophils was 0.9; the maturation index of the protoplasm of the erythro- and normoblasts.0.8. The mitotic activity of the cells of the erythroblastic series was 5 : 500, that of the leukoblastic series.2 : 500.

With the decrease in the number of cells in the marrow obtained by puncture, observed during the days following puncture, the relative cell proportions shown in the myelogram were considerably disturbed. In the first place there was an appreciable fall in the percentage of immature cells of the neutrophil series (promyelocytes, myelocytes, and juvenile neutrophils) and an appreciable rise in the percentage of mature neutrophils (from 28.6% to 41.1%), lymphocytes (from 9.7% to 25.7%) and also of monocytes (from 1.1% to 2.4%). The proportion of cells of the red series was reduced, and the mitotic activity fell – the number of mitoses in cells of the red series was 2.1 : 500, and of the white series, 1.5 : 500.

The functional impairment of hemopoiesis is made apparent by determination of the leuko-erythroblastic ratio (7.3) and the maturation index of the neutrophils (0.3). Characteristic cells, often in groups of 3-6 at a time, are seen in large numbers in the films at this period, and by their morphology they may be classed as osteoblasts. The appearance of osteoblasts in the marrow specimens is most evident in the 6th-7th week of puncture of the bone marrow, and later their number decreases. Another characteristic feature is that the marrow obtained after frequent aspiration was in no way distinguishable in its external appearance from normal, especially by the presence of a large number of lipoid scintillations on its surface.

Changes were also observed in the peripheral blood indices. As the number of punctures increased (to the eight week), the qualitative composition of the blood was modified, as follows: young marrow cells of the type of hemocytoblasts, promyelocytes, myelocytes, reticulum or plasma cells, binuclear lymphocytes, and also erythroblasts, polychromatophilic cells, and erythrocytes with Jolly bodies began to appear in the peripheral blood. The presence of a large number of bare nuclear cells and of cells in a state of leukocytolysis was also noted; all these changes in the blood were clearly shown in the monkeys Koster and Anyb.

It thus follows from the findings described that repeated aspiration from the same area of the bone marrow leads to profound disturbance of the processes of hemopoiesis taking place in that area. This must be taken into consideration when the dynamics of the cellular composition of the bone marrow is being studied.

SUMMARY

The changes in the cellular composition of the bone marrow were studied in 5 monkeys of the species Papio hamadryas. The bone marrow was obtained from the tibial tuberosity of both extremities with the aid of I. A. Kassirskii's needle. Experiments were conducted for a period of 6-9 months in the morning hours, before feeding the animals, once a week. The absolute number of leukocytes in the punctate decreased as a result of frequent punctures; the normal cell ratio in myelograms was disturbed. Qualitative changes also occurred in the peripheral blood. The changes observed were very stable and persisted for a long time.

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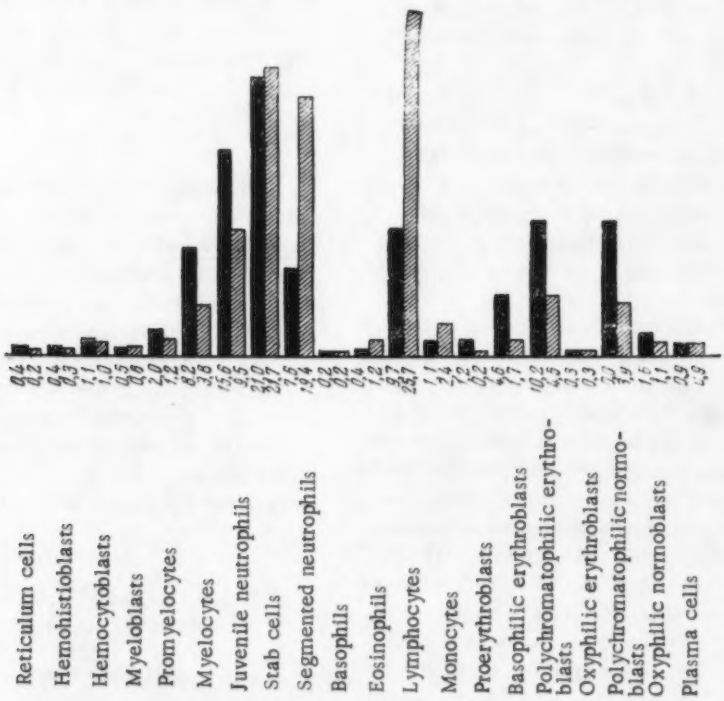

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A CAPILLARY MERCURY–ALKALINE PICK-UP WITH AIR DAMPING FOR RECORDING ACCELERATION BALLISTOCARDIOGRAMS

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(Presented by Active Member AMN SSSR V. V. Parin)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 116-118, August, 1960

Original article submitted August 17, 1959

Ballistocardiography is one of the methods of recording the propulsive function of the heart [1, 2]. Existing pick-ups are of several types, but nearly all are based on the electromagnetic or photoelectric principle. In recording the acceleration ballistocardiogram, pick-ups of this type require differentiation of the output signal. If this condition is observed, however, they record the mean values between velocity and acceleration [3]. There are reports in the literature of the use of a pick-up based on the so-called "U effect" for recording the ballistocardiogram. This system consists of a capillary tube, filled with alternate drops of mercury and 0.1 N sulfuric acid. According to Watanabe, Elliot and Packard, and Bodrogi, this pick-up enables a ballistocardiogram of pure acceleration to be recorded (cited by Bodrogi [3]). Trials of pick-ups constructed by this method have shown that, on a scale suitable for clinical purposes, they possess an essential drawback – the low value of the output signal demands high amplification.

In our experiments we introduced a series of new and essential additions into the construction of a pick-up of this type, which serve to overcome this drawback, and thereby made it more suitable for clinical and experimental work.

The capillary tube was filled with alternate columns of mercury and alkali (0.75 N NaOH solution). This greatly increased the sensitivity of the pick-up by comparison with an Elliot-Packard-Bodgori pick-up of the same size. Observations showed that the magnitude of the electromotive force created by mechanical displacement of the pick-up was greater, the more freely the drops of mercury moved. This led us to the idea of creating an air damper between each pair of drops (mercury–alkali). A system of this type increased the output signal in general eight to tenfold, and enabled the ballistocardiogram to be recorded with only slight modifications of the pick-up on the ÉKP-4 electrocardiograph, providing a sensitivity of the apparatus of 0.2 mv.

We developed two variants of the pick-up. The first variant was a capillary tube 1 mm in diameter (from a Panchenkov apparatus) and 15 cm long, filled with alternate columns of mercury and alkali and air bubbles. The length of each column of mercury was 6 mm, of alkali 2 mm, and the space occupied by the air bubbles was 1 mm. The number of columns of mercury was 7-15. The end portions of the capillary tube were filled with mercury, into which dipped copper wire electrodes 0.5-1.0 mm in diameter, after which the ends of the capillary tube were sealed with Mendeleev's putty. The outline of the first variant of the pick-up, which was quite convenient to work with, is illustrated in Fig. 1. The curves obtained with its aid were completely indistinguishable from the curves reproduced in the literature. A defect of this type of construction is the instability of the pick-up to sudden mechanical influences in a longitudinal direction. Accordingly, we developed a second variant: in its construction we introduced an additional device which made the pick-up stable to sudden mechanical influ-

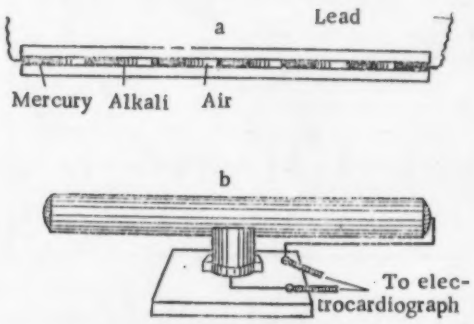

Fig. 1. Scheme of the mercury-alkaline pick-up for recording the acceleration ballistocardiogram (first variant). a) Diagram of the filled capillary tube; b) general view of the assembled pick-up.

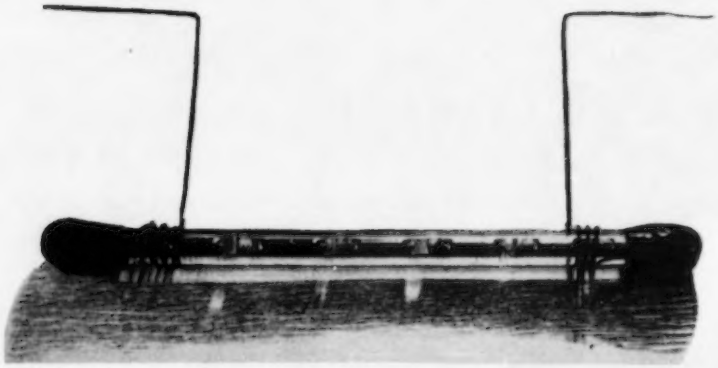

Fig. 2. Mercury-alkaline pick-up for recording the acceleration ballistocardiogram (second variant).

ences directed along its axis. We used a capillary tube 3 mm in diameter; into this tube, between each pair of dissimilar drops (mercury–alkali) and air bubble, we inserted a penoplast plug, which restricted the displacement of the drops of mercury within the confines of the space thus created (Fig. 2). This type of construction, without altering the physical parameters of the pick-up, ensures complete stability to sudden mechanical jolts.

In order to fill the capillary tube more accurately and quickly, we used a specially constructed apparatus, the main part of which was a 2 ml syringe. The capillary tube, however, may also be filled by means of an ordinary syringe, fixed in a stand. In doing so, it is essential that the entire system, including the syringe, connecting the rubber tube and the capillary tube itself, should first be filled with the alkaline solution. The capillary tube is filled from a test tube (into which mercury and alkali are poured in two layers), by placing its end first in the mercury and then in the alkaline solution, and afterward in the air. In the second variant of the construction a thick capillary tube was selected and filled by means of an ordinary pipette. It is advisable to mount the complete pick-up in a copper or brass tube, and to fix it on a plate of organic glass. The tube protects the apparatus against outside forces, and at the same time acts as a screen.

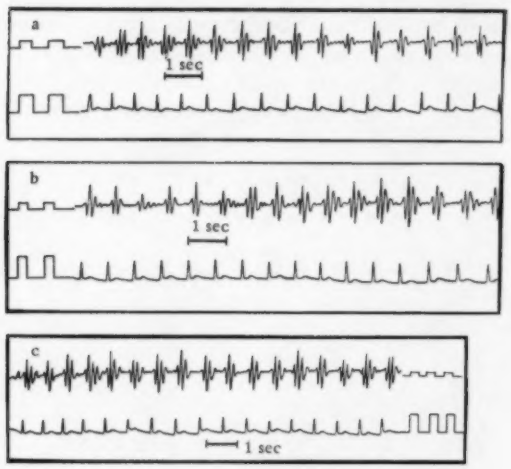

Fig. 3. Acceleration ballistocardiograms recorded simultaneously with electrocardiograms from the leg of a healthy human subject by means of the mercury–alkaline pick-up. Velocity of movement of the tape 2 cm/sec. a, b) Curves recorded with the use of a 0.03 mf filtrate; c) curves recorded without a filtrate.

Investigation of many pick-ups showed that their intrinsic frequency depends on the mass of mercury and the number of its columns. The greater the mass of the mercury, the lower the frequency of oscillation. This enables each researcher to produce a pick-up with a frequency most suited to his needs – from 65 to 300 cps. The apparatus described is compact and convenient in use and enables the ballistocardiogram to be recorded with the subject in any position and from any point on the body. The pick-up is simple in construction and may quickly be made in any medical institution. The weight of the filled capillary tube is 13-20 g, and that of the assembled pick-up is 130-150 g. The recording of ballistocardiograms taken with the capillary mercury–alkaline pick-up in healthy human subjects and patients showed the high sensitivity of the apparatus to changes in the mechanical working of the heart. The character of the curves, the amplitude of the waves, and the time relationships of the wave segments were identical to those reported in the literature.

In Fig. 3 are shown ballistocardiograms, recorded with the capillary mercury–alkaline pick-up (second variant) from the leg of a healthy human subject.

SUMMARY

The paper gives a description of the design, and presents physical characteristics, of a capillary mercury–alkaline pick-up with air damping, as well as the procedure used in its operation. It is based upon the principle known as the "U effect," and consists of a capillary tube filled with alternating columns of mercury, alkali, and air. Between each pair of adjoining drops (mercury, alkali) there are provided stops in the shape of penoplast plugs to raise the mechanical stability of the pick-up. The natural frequency of the pick-up may be within the range of 65-300 cycles per sec. The pick-up supplied with mechanical stops is stable and gives curves identical to those described in the literature.

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A PUMP-FLOWMETER FOR RECORDING THE VOLUME VELOCITY OF THE CIRCULATION

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(Presented by Active Member AMN SSSR V. V. Zakusov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 50, No. 8, pp. 118-120, August, 1960
Original article submitted October 12, 1959

The pump-flowmeter constructed in the manner suggested below enables the volume velocity of the venous return blood flow to be measured, and at the same time, it pumps the outflowing venous blood back into the animal's blood stream. This pump has no aspirating action and merely removes the blood which enters it.

The scheme of the apparatus described is shown in Fig. 1. It consists of the following parts: two working heads A and B, working in turn; three external electromagnetic valves C, D, and E; a measuring cylinder F; a water bath G, and a feed for the pump H, consisting of a Mariotte's jar.

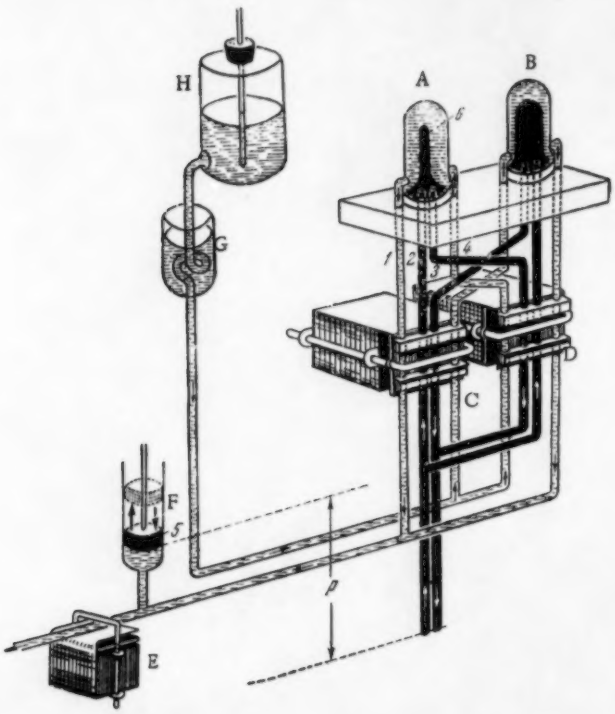

Fig. 1. The pump-flowmeter. Explanation in text.

We begin the description of the full cycle of operation of the pump at the head A. When the valve C is open, valve D is closed; under these circumstances blood from the peripheral segment of the vein passes along the tube 2 into a thin-walled rubber bag 6, situated inside the working head A. The inside of the chamber of the working head is filled with water. Blood entering the rubber bag 6 displaces an equivalent volume of water from the working head A into the measuring cylinder F through the tube 1. Immediately after closure of the valve C, the valve D opens, and water from the vessel H passes under pressure along the tube 4 into the interior of the working head A, displacing the blood contained in the rubber bag 6 through the tube 3 into the central segment of the vein. After closure of the valve D, valve C again opens, and the cycle is repeated. Impulses to the electromagnetic valve pass from a distributor mounted on a Warren motor (2 rpm).

The working head B acts in sequence with the head A. When the valve D is open and valve C closed, blood passes into the head B, and when valve C is open and valve D closed, blood is expelled from the head B. Thus when blood enters one head, it leaves the other, and vice versa. By this means it is possible to obtain a continuous, pulsating flow of blood through the apparatus. Water passes into the measuring cylinder in a volume precisely equal to the volume of blood passing through the pump in unit time. Water displaced from the working heads when the valve E is closed fills the measuring cylinder and lifts the float 5, the movement of which is recorded by means of a light lever on the drum of a kymograph. The valve E is closed periodically for a definite period of time. Under these conditions the height of elevation of the float is determined by the volume of blood passing in unit time through the

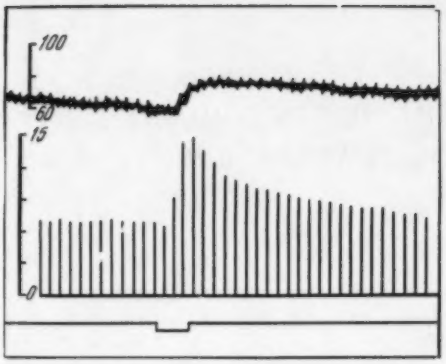

Fig. 2. The effect of sodium salicylate (50 mg/kg) on the volume velocity of the blood flow from the coronary sinus. Significance of the curves (from above down): arterial pressure in mm Hg; outflow of blood from the coronary sinus in ml/min; marker of administration of the drug. Measurement of the velocity of the blood flow was made every 30 seconds.

pump. When the valve E opens, the cylinder is emptied. When the valve E is closed, the cycle is repeated. The working of the valve E is not connected with the action of the valves C and D.

It should be mentioned that the pressure on the inlet of the tube 2 is determined by the difference between its level and the level of the measuring cylinder, indicated in the figure by P. In order to make the conditions of flow of the blood into the flowmeter close to physiological, this distance P must be equivalent to the value of the venous pressure in the vessel which is being investigated.

Using the apparatus described, we measured the volume velocity of the blood flow from the coronary sinus of the cat's heart. For this purpose the tube 2 (see Fig. 1) was linked to a polyethylene catheter inserted into the coronary sinus, and the tube 3, to the central end of the jugular vein. A recording of the volume velocity of the blood outflow is shown in Fig. 2. The blood flow was measured every 30 seconds. Movement of the kymograph drum was effected only in the intervals between the measurements of the velocity of the blood flow, so that the results of each measurement appear in the form of a vertical line. The higher the velocity of the blood flow, the higher the line on the recording.

SUMMARY

A new design for a pump-flowmeter is suggested, with which it is possible to measure the volume velocity of the venous blood outflow and to simultaneously pump the outflowing venous blood into the animal's blood stream. The pump has no suction action and only removes the blood entering it.

A SIMPLE METHOD OF SIMULTANEOUS GRAPHIC RECORDING OF THE TONE, MOTOR ACTIVITY, AND EVACUATORY FUNCTION OF THE STOMACH

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 120-122, August, 1960

Original article submitted December 20, 1958

Several methods of study of the motor function of the stomach are in regular use – roentgenological, electrographic, and manometric. The simplest and most widely used method of manometric recording of the motor activity of the stomach is that developed in work by V. N. Boldyrev [1], V. V. Gerbst [3], N. N. Savitskii [6], I. T. Kurtsin [5], and others, which played an important part in the study of the motor function of the stomach and intestine. The method used by these authors, however, does not enable the simultaneous permanent recording of the characteristics of all aspects of the motor activity of the stomach. In fact, these researchers studied only the periodic motor activity of the stomach or its peristaltic contractions by means of the introduction of a rubber balloon into that organ.

I. I. Veretyanov [2] suggested a convenient and simple method, which consisted of attaching a thin catheter, introduced into the stomach, to a Marey's capsule, and recording the peristaltic contractions of the stomach when it contained a liquid test breakfast. To determine the evacuatory power of the stomach, however, this author recommends recording the motor activity of the stomach for only 15 minutes, and then extracting and measuring the gastric contents. In the study of the tone of the stomach, no graphic recording was undertaken.

Gaultier [4] estimated the tone of the stomach by inflating the organ with air and measuring the volume and pressure of air introduced. Weitz [7] used water for this purpose.

The evacuatory power of the stomach is usually studied by means of the extraction of the residual gastric contents at different times, or by means of roentgenoscopy.

In order to obtain simultaneous graphic recordings of the tone, the peristalsis and the evacuatory function of the stomach in dogs with a gastric fistula, we suggest the following method. To the fistula a system is hermetically connected (Fig. 1), a system consisting of a funnel 1 (volume 2-3 liters), mounted on a rigid stand 3, and a rubber tube 2 (diameter 1.5-2.0 cm). Before the experiment commences, the funnel is filled with 0.5-2.0 liters of broth, milk, carefully minced bread, and meat mixed with a large quantity of broth, and so on. The dog may also be fed with its usual breakfast, while at the same time broth is introduced into the stomach through the tube and funnel.

At this time the rubber tube must be clamped with the clamp 4 (a second clamp is indicated by number 6). A glass tube 5 (diameter 0.3-0.5 cm) is immersed in the liquid down to the level of the lower margin of the gastric fistula. As a preliminary step the tube 5 is hermetically connected by means of the rubber tube 7 to a Marey's capsule 8 mounted on a universal stand 9. The ink-writing instrument 10 in this way is inclined upward to a considerable degree. A time marker 11 with 5-second intervals is included, and the kymograph 12 is set in motion for a large number of revolutions. A paper strip is placed on the kymograph and extension.

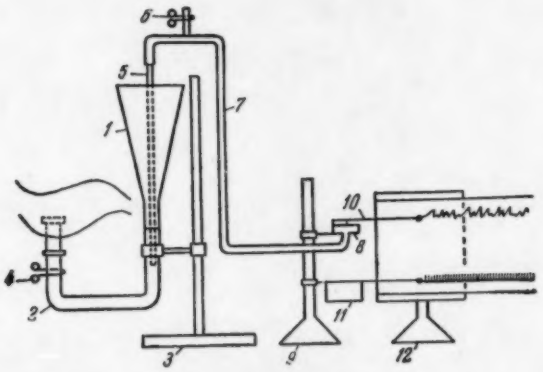

Fig. 1. Scheme of the apparatus for the simultaneous recording of the tone, motor activity and evacuatory function of the stomach.

After removal of the clamp 4, allowing free passage of fluid from the funnel into the stomach, the pen descends more or less rapidly, describing a broken curve, and it then begins to record the peristaltic contractions of the stomach, transmitted to the recording system through the column of fluid. The peristaltic contractions shown on the kymogram gradually give way to a series of high, protracted waves; the movements of the stomach then cease. The general appearance of the curve is shown in Fig. 2.

The time of arrival of the liquid in the stomach, recorded on the kymogram as a curve of fall of the pen (see Fig. 2, cut A-B), characterizes the tone of the stomach during its distension by the large volume of liquid. This function may later be estimated by the height of the recording of the peristaltic contractions in relation to the horizontal line drawn on the kymogram, which is usually the line of the time marker.

Special experiments (measurement of the volume of the gastric contents at various times during the experiment, recording of the motor activity during the free outflow of liquid from the open duodenal fistula) showed that the first waves –which were large and protracted in time–characterizing the contractions of the stomach (see Fig. 2, C, D, E) correspond to the moment of evacuation of the greater part of the gastric contents; the last wave of this type (see Fig. 2, E), together with the appearance of small, gradually lessening contractions on the kymogram or with the cessation of all contractions of the stomach, indicates the end of the transfer of all the gastric contents into the intestine.

By the use of the method described, and keeping constant the size of the writing arm, the state of the Marey's capsule, the level of immersion of the glass tube in the liquid, the position of the funnel on the stand, and the volume of liquid introduced, the following indices of the motor activity of the stomach can be obtained:

1) the time taken by the liquid to pass from the funnel into the stomach (in seconds), characterizing the tone of the stomach when distended with fluid;

2) the level of recording of the peristaltic contractions of the stomach in relation to the horizontal line of the time marks (in mm), determining the tone of the stomach in the course of the experiment and the dynamics of its evacuation;

3) the number of contractions of the stomach, the amplitude of the waves (in mm) reflecting the contractions, and the coefficient of motor activity of the stomach (i.e., the ratio between the duration of the sum total of the contraction waves and the duration of the sum total of the pauses between them) – values which characterize gastric peristalsis;

4) the time taken by the gastric contents to pass into the intestine (in minutes), giving an indication of the evacuatory power of the stomach.

The indices referred to in items 2 and 3 should be determined in successive intervals of 15-30 minutes.

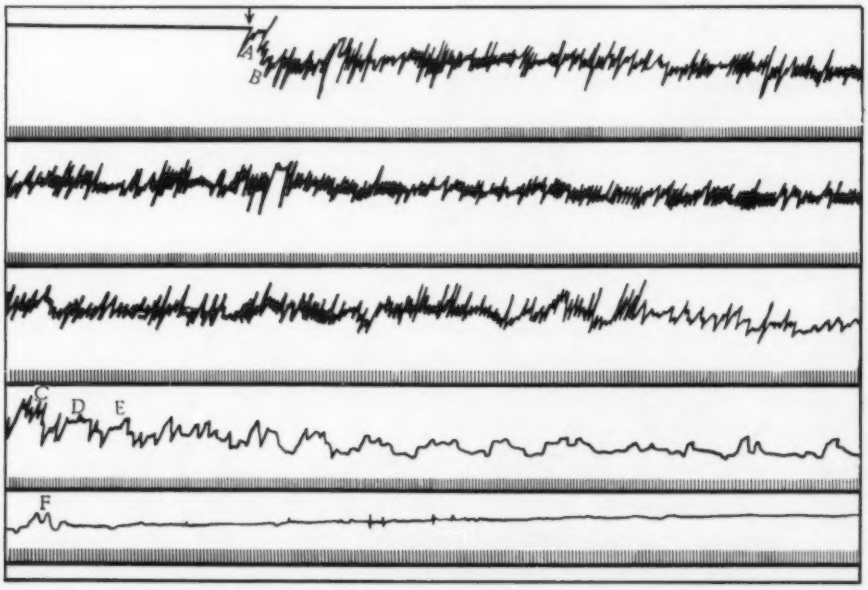

Fig. 2. Recorded curve of the tone, peristalsis and evacuatory power of the stomach after introduction of 1 liter of broth (dog Alpha). Explanation in text.

SUMMARY

Following introduction into a dog's stomach of 0.5 to 2 liters of fluids (broth, milk, mashed bread and minced meat in a large quantity of broth), a simple system, based on the principle of connected vessels, enables simultaneous graphic recordings to be made of the tone and peristalsis of the stomach, as well as of the time necessary to achieve complete evacuation of the gastric contents.

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COUNTING THE FREQUENCY OF MUSCLE ACTION CURRENTS ON THE KYMOGRAPH

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 122-124, August, 1960

Original article submitted September 3, 1959

The functional state of muscles is determined electrophysiologically by the frequency and amplitude of their action currents, recorded by means of an amplifying and oscillographic apparatus. The principal difficulty in the interpretation of the myograms obtained by this method is that the number of myographic impulses is not easily counted, especially when it is large.

One way of overcoming these difficulties would be by counting the impulses automatically and recording them by means of an apparatus of the PS-64 type. Counters of the PS-64 type are incorporated in certain forms of biophysical apparatus for measuring the radiation from labeled atoms.

When a definite number of electrical impulses is fed into the input of a counting device, a smaller number of impulses is produced at its output in accordance with the particular coefficient of division. For example, when the coefficient of division of the counting system is 64, the apparatus will record every 64th impulse reaching the input of the apparatus. The bioelectrical potentials arising in a muscle must, of course, be first amplified to a value sufficiently large to be fixed by the receiving part of the PS-64 apparatus.

The assembly scheme of such an apparatus is shown in Fig. 1. Action currents of a muscle after amplification (coefficient of amplification of the order of 100,000-200,000), are passed to a PS-64 apparatus, the output of which operates an electromagnetic decimal counter and relay, which sets in motion an electromagnetic marker.

By means of this apparatus it is possible to count the sum total of bioelectrical impulses during a given interval of time, corresponding to various functional states of the muscle, which gives the characteristics of frequency changes, and enables them to be differentiated in time. Impulses whose amplitude after amplification lies below the threshold of operation of the PS-64 apparatus will not, of course, be counted. Practice has shown, however, that this is not an essential failing, and the layout of the apparatus in the form in which it is shown is suitable for the investigation of the work of muscles. This method has been found valuable in the study of hyperkinesis [2].

By way of example of the suitability of the method for the study of the bioelectrical activity of muscles during static and dynamic work, we give the results of our observations, made jointly with A. K. Troshin, on a human subject (a youth of 16 years), in a state of deep hypnotic sleep. In the region of the right biceps brachii muscle were applied two plastic electrodes, 10 mm in diameter, with cotton applicators soaked in a 5% solution of common salt. The electrodes were connected to a counting device (coefficient of division 64) and a kymograph (see Fig. 1).

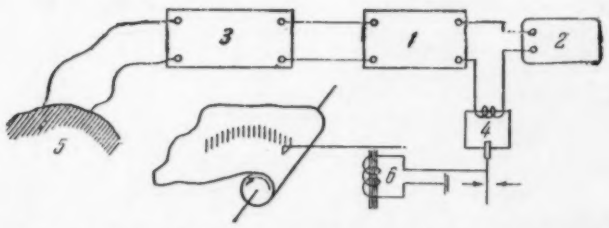

Fig. 1. Layout of the apparatus for counting and recording the bioelectrical impulses of muscles. 1) Counter PS-64; 2) impulse counter SB-1 m/100; 3) amplifier; 4) type RP-7 relay; 5) muscle; 6) kymograph marker.

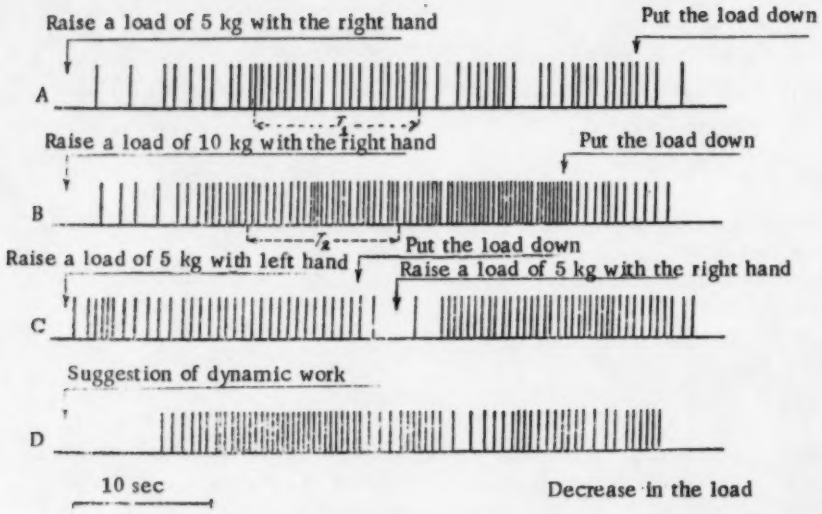
Raise a load of 5 kg with the right hand
Put the load down
A
Raise a load of 10 kg with the right hand
Put the load down
B
Raise a load of 5 kg with left hand
Put the load down
Raise a load of 5 kg with the right hand
C
Suggestion of dynamic work
D
10 sec
Decrease in the load

Fig. 2. Kymograms of the bioelectrical impulses from the biceps muscle in man, recorded by means of a PS-64 electronic counting system.

On the kymogram (Fig. 2) are shown the variations in the frequency of the bioelectrical impulses. During tranquil hypnotic sleep impulses were absent. On kymograms A and B may be seen the differential response to suggested static work of differing severity. The tracing C was obtained during the suggestion to the subject that he carry out static work, first with the left hand and then with the right (the action currents were taken as before from the right biceps brachii muscle). The tracing D illustrates the bioelectrical effect during the suggestion to the subject that he carry out dynamic work (work the mincer with the right hand). A reduction in the frequency of the impulses coincided with the suggestion of lightening the load.

The true value of the frequency of the impulses for any interval of time is determined by taking the product of the number of marks and the coefficient of the counting system (in this case 64). The frequency of the bioelectrical impulses in response to the suggestion, "raise 5 kg," during an interval of time $T_1 = 10$ seconds, is thus $(21 \times 64)/10 = 134.4$ imp/sec. For the interval of time T_2, corresponding to a twofold increase in the load, the frequency of the action currents of the muscle is $(28 \times 64)/10 = 179.1$ imp/sec.

SUMMARY

The author describes a practical method of analyzing the frequency of muscle action currents, based upon the use of the widely employed computer PS-64 and of other apparatus.

The method suggested makes it possible not only to analyze the frequency changes of the bioelectric effect on the muscles, but also to measure the latent periods in various transitional conditions of the muscles—at rest and during work.

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ASSESSMENT OF THYROID GLAND FUNCTION IN RATS BY RADIOACTIVE IODINE ABSORPTION

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 8, pp. 124-126, August, 1960
Original article submitted August 14, 1959

The determination of the functional state of the thyroid gland by means of radioactive iodine in chronic experiments on rats is of considerable importance, for rats are a convenient subjectfor experimental investigation on a large scale.

The assessment of the function of the thyroid gland by means of radioactive iodine is complicated in these animals by the difficulty of keeping them during the required period of time in the appropriate position in relation to the counter, which interferes with observations on the trend of iodine absorption by the thyroid gland.

We have invented a device for fixation of rats, which has made it possible to determine the quantity of radioactive iodine absorbed by these animals during the course of prolonged experiments, by means of the Geiger-Muller counter.

This device is shown in Fig. 1, and consists of a base – a wooden board (1) measuring 200-400 mm, covered with tinfoil –to the front edge of which, the bed for the screen housing of the MS-4 counter tube (2) is rigidly fixed.

The counter tube is enclosed in a lead tube, 47 mm in diameter, with a wall 12 mm in thickness. In the middle part of the tube, and for half its diameter, a rectangular piece is cut out to a width of 40-45 mm (3), in which the rat's neck is placed. Since the counter tube is in the center of the lead tube, its upper half projects above the base of the cut-out portion, which guarantees firm contact between the counter and the anterior surface of the animal's neck.

A small cage (4) for the rat is placed next to the counter, screened as shown, at the site of the cut-out part of the tube, and is fixed to the baseboard by means of nails. The cage also has a base – a wooden board measuring 300 × 50 mm, covered with tin. Starting at the front edge and for a distance of 150 mm, a number of flat metal arches (5) are fixed to this base, and make the wire of the cage sufficiently firm. The animal is placed in the arched space thus formed.

A series of holes for escape of urine (6) is made in the base of the cage, in the front part near the arch. In the posterior part, holes are made in the midline of the base at intervals of 10 mm for fixation of the posterior, movable door of the cage by means of a bent pin (7).

To the front arch is attached a transparent plastic cap (8), bent to the shape of the animal's neck (in the form of half a truncated cone). The attachment of this cap is hinged, so that its height can be regulated by means of a special flat lever (9).

Movement of the rat in an anteroposterior direction is prevented in front by means of a special stop, consisting of a metal cone with holes, which is attached by means of shackles to the edge of the cut-out portion of the lead housing of the counter tube (10), and at the

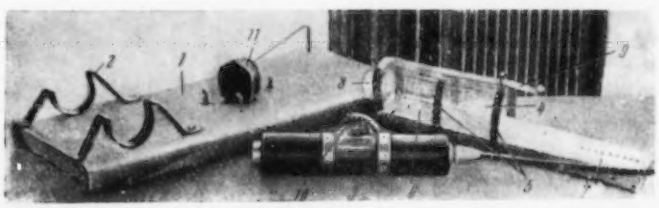


Fig. 1. Device for fixation of rats (in unassembled view). For explanation see text.

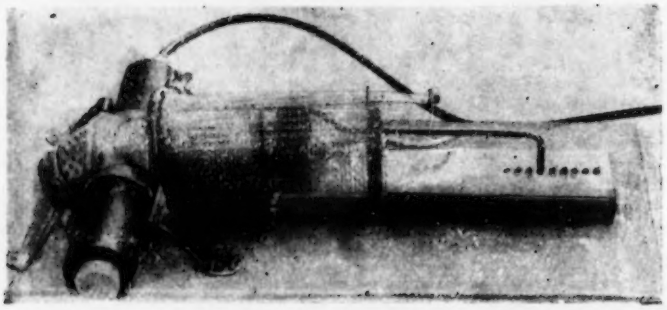


Fig. 2. General view of the device for fixation of rats with an experimental animal in situ.

back by a movable wall (11), whose position is regulated to conform to the size of the rat, and is fixed by means of a pin in a hole in the base of the cage. A portion of the lower part of the posterior wall is cut out to accommodate the rat's tail.

In order to place the rat in the cage, the posterior wall is removed, the animal is placed inside by means of tongs, and the movable posterior wall is pushed up to the animal. The rat moves forward and puts its head out under the cap. In this position the posterior wall of the cage is fixed, and the cap is lowered by means of the lever. The cap must fit firmly to the rat's neck and to the counter, while its front edge supports the head behind the ears. As can be seen in Fig. 2, the rat's head is outside the cap, and in these conditions the rat can freely breathe and perform slight movements of the head.

Escape of the rat from the cage is prevented by the constricted end of the cap and the front stop, which partially covers the head without compressing it.

This apparatus has been tested on a large number of rats.

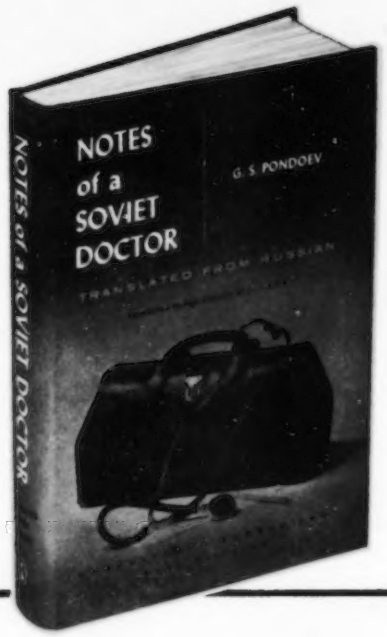
Observations on the dynamics of iodine absorption were conducted on the same animals for 3 days. Counting was undertaken every 2 hours after intraperitoneal injection of the isotope. During the counting the rat was present in the cage for 30 minutes. During this time 2-3 separate determinations were made.

As our results showed, during each of these determinations the number of impulses counted per minute in this interval of time (30 minutes) differed only very slightly from the others, thus demonstrating the stability of the position of the rat's thyroid gland in relation to the counter, and enabling curves to be drawn to show the absorption of iodine by the thyroid gland.

SUMMARY

A device for fixation of white rats is described. It aids in determining the quantity of radioiodine absorbed by the thyroid gland in chronic experiments (by means of the Geiger-Muller counter).

This adjustment was tested on many rats. Observations of iodine absorption dynamics were conducted for a period of three days in the same animals. This device guarantees stability of the thyroid gland with respect to the counter and enables the curves of iodine absorption by the thyroid gland to be plotted.



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